PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 39/395, G01N 33/577, 33/68 — A1

(11) International Publication Number: **WO 95/07716**

(43) International Publication Date: 23 March 1995 (23.03.95)

(21) International Application Number: PCT/EP94/03114

(22) International Filing Date: 16 September 1994 (16.09.94)

(30) Priority Data:
93402279.9 17 September 1993 (17.09.93) EP
(34) Countries for which the regional or international application was filed: AT et al.

(71) Applicant *(for all designated States except US)*: LABORATOIRE EUROPEEN DE BIOTECHNOLOGIE S.A. [FR/FR]; 30, avenue George V, F-75008 Paris (FR).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: TOVEY, Michael, G. [GB/FR]; 7, rue Lagrange, F-75005 Paris (FR). BENIZRI, Eric, Jacob [FR/FR]; 34, boulevard de Cimiez, F-06000 Nice (FR).

(74) Agents: GUTMANN, Ernest et al.; Ernest Gutmann-Yves Plasseraud S.A., 3, rue Chauveau-Lagarde, F-75008 Paris (FR).

(81) Designated States: AU, BB, BG, BR, BY, CA, CN, CZ, HU, JP, KP, KR, KZ, LK, LV, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PHARMACEUTICAL COMPOSITION COMPRISING MONOCLONAL ANTIBODIES AGAINST THE INTERFERON RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST TYPE I INTERFERON

(57) Abstract

The invention relates to pharmaceutical compositions for use as immunomodulator, comprising a purified preparation of a monoclonal antibody directed against the human interferon class I receptor (IFN-R) characterized by the following properties: it recognizes the extracellular domain of the human IFN-R, and it has a neutralizing capacity against the biological properties of the human type I-IFN. It further concerns their use for the diagnosis.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

PHARMACEUTICAL COMPOSITION COMPRISING MONOCLONAL ANTIBODIES AGAINST THE INTERFERON RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST TYPE I INTERFERON

The interferons (IFN) constitute a group of secreted proteins which exhibit a wide range of biological activities and are characterized by their capacity to induce an antiviral state in vertebrate cells (I. Gresser and M.G. Tovey Biochem Biophys. Acta 516:231, 1978). There are three antigenic classes of IFN : alpha (α), beta (β) and gamma. IFNα and IFNβ together are known as the type I interferon.

Natural type I human interferon comprises 16 or more closely related proteins encoded by distinct genes with a high degree of structural homology (Weissmann and Weber, Prog. Nucl. Acid. Res. Mol. Biol. 33:251, 1986).

The human IFNα locus comprises two subfamilies. The first subfamily consists of 14 non allelic genes and 4 pseudogenes having at least 80% homology. The second subfamily, αII or omega (ω), contains 5 pseudogenes and 1 functional gene which exhibits 70% homology with the IFNα genes (Weissmann and Weber 1986).

The subtypes of IFNα have different specific activities but they possess the same biological spectrum (Streuli et al. PNAS-USA 78:2848, 1981) and have the same cellular receptor (Agnet M. et al. in "Interferon 5" Ed. I. Gresser p. 1-22, Academic Press, London 1983).

The interferon β (IFNβ) is encoded by a single gene which has approximately 50% homology with the IFNα genes.

The interferon α subtypes and interferon β bind to the same receptor on the cell surface.

The interferon gamma (IFN gamma) is also encoded by a single copy, which has little homology with the IFNα and IFNβ genes. The receptor for IFN gamma is distinct from the receptor of the α and β interferons.

For the purpose of the present invention the receptor of α and β classes of IFN will be designated IFN-R. This represents natural type I receptor. The group of proteins forming natural interferon α will be designated IFNα, and type I-IFN will represent both natural IFNα, IFNω, and IFNβ.

Despite the fact that interferon is a potent antiviral agent, there is considerable evidence to suggest, that many of the characteristic symptoms of acute virus diseases such as upper respiratory tract infections are caused by an overproduction of interferon alpha. Furthermore, IFN alpha has been shown to contribute to the pathogenesis of certain chronic virus infections in experimental animals and the available evidence suggests that this is also the case for certain human chronic virus diseases such as those due to measles virus.

The interferons α are also potent immuno-regulatory molecules which stimulate polyclonal B-cell activation, enhance NK cell cytotoxicity, inhibit T-cell functions, and modulate the expression of the major histocompatibility complex (MHC) class 1 antigens, all of which are implicated in the induction of autoimmunity and in graft rejection. The abnormal production of interferon α is associated with a number of autoimmune diseases, immune deficiencies and inflammatory disorders including systemic lupus erythematosus (SLE), type I diabetes, psoriasis,

rheumatoid arthritis, multiple sclerosis, Behçet's disease, aplastic anemia, the acquired immunodeficiency syndrome (AIDS) and severe combined immunodeficiency disease. The presence of interferon α in the serum of patients with systemic lupus is correlated with both the clinical and humoral signs of increased disease activity. The production of interferon α in HIV positive subjects is also highly predictive of disease evolution.

Especially a close correlation also exists between the presence of circulating acidolabile interferon α and disease progression in patients with the acquired immunodeficiency syndrome (AIDS) (Mildvan D. et al. 1992, The Lancet 339:353).

Administration of interferon α has been reported to exacerbate underlying disease in patients with psoriasis and multiple sclerosis and to induce a SLE like syndrome in patients without a previous history of autoimmune disease. Interferon α has also been shown to induce glomerulonephritis in normal mice (Gresser et al., 1976, Nature, 263:420) and to accelerate the outset of the spontaneous autoimmune disease of NZB/W mice (Adam et al., 1980, Clin. Exp. Immunol., 40:373).

The constitutive expression of interferon α in the pancreas of transgenic mice has also been shown to lead to the development of insulino-dependent diabetes the development of which is abrogated by treatment of the animals with specific antibody to interferon α (Stewart et al., 1993, Science, 260:1942).

Treatment of lethaly irradiated mice with interferon α has also been shown to enhance resistance to allogenic bone marrow grafts while treatment with antibody to inteferon α was found to inhibit rejection (Affifi et al., 1985, J. Immunol. 134:3739). Treatment

of rats with antibody to interferon α/β has also been reported to prolong cardiac allograft survival in these animals (Gugenheim et al., 1992, Transplant. Int. 5:460).

Interferon α is also produced during the course of graft-versus-host disease (GVHD) in parallel with the enhanced NK cell activity characteristic of systemic GVDH. Interferon α is the principal modulator of NK cell cytotoxicity and administration of interferon α has been shown to enhance the intestinal consequences of GVDH in normal mice (Cleveland et al., 1987, Cell Immunol. 110:120).

The object of the present invention is to provide new antagonists against the biological activities of the human type I-IFN. These antagonists could be used for therapeutical, including prophylaxis purposes, in cases where the type I-IFN (IFN α/β)is abnormaly produced and when this abnormal production is associated with pathological symptoms. Such antagonists could also be used for the diagnosis of various diseases or for the study of the evolution of such diseases.

In order to define such antagonists, the inventors have taken into account the fact that human natural type I-IFN is in fact constituted of a mixture of interferons (subspecies) and the fact that the composition of this association of different subtypes of interferons varies both quantitatively and qualitatively.

Some natural interferons, such as the ones secreted by Namalwa cells (Namalwa interferon) or leukocyte (leucocyte interferon) have been studied in detail (N.B. Finter and K.H. Fautes, Interferon 2, 1980, p. 65-79 I. Gresser Editor Academic Press ; K.

Cantell et al, Interferon 1, 1979 p. 2-25, I. Gresser Editor Academic Press) and were used by the inventors to define natural type I interferons.

In some pathological cases, like AIDS, interferons having some special properties have been described (O.T. Preble et al, Annals of New-York Academy of Sciences p. 65-75). This interferon involved in pathological cases like AIDS nevertheless binds to the same receptor, as described above.

One object of the present invention is to provide an antagonist of the type I-IFN, which would be able to inhibit or neutralize, to a determined extent, the biological properties of the human type I-IFN, that is to say, to neutralize in vivo a mixture of α, β, ω subspecies.

These antagonists can be utilized in the composition of drugs as immunomodulators and their characterization has been rendered possible by the identification by the inventors, of the nature of the interferon occuring in various pathologies and also by the development of animal models.

The present inventors, having developed appropriate in vivo animal models, have been able to demonstrate that some monoclonal antibodies are capable to interact in vivo on the immune response, with the type I-IFN receptor, in a therapeutically efficient manner as regards various pathologies.

Especially the inventors have been able to define conditions where the in vivo administration of monoclonal antibodies directed against the type I IFN receptor can interact and in some cases inhibit symptoms of viral diseases resulting from viral infections, especially infections due to the HIV or HTlV retroviruses, or can inhibit allogenic graft

rejection or can inhibit the effect of Graft Versus Host Disease.

These immunomodulators will be utilized as such for the treatment of diseases involving abnormal production of interferon, or will be utilized in combination either simultaneously or separately, with other known immunomodulators or drugs especially viral drugs.

For instance, the inventors have shown that in some situations monoclonal antibodies directed against the type I IFN receptor can have a useful synergic effect with other therapeutic agents. This appears to be the case for instance when monoclonal antibodies against type I IFN receptor are associated with an immunosuppressive drug like cyclosporin A.

Accordingly the inventors have defined compositions comprising antibodies, especially monoclonal antibodies, which have the property of being antagonists to the type I-IFN due to their activity as immunomodulators. These antibodies are directed against the human type I-IFN receptor.

The invention thus relates to the use of a preparation of purified monoclonal antibodies in pharmaceutical compositions, for use as immunomodulator, for the in vivo treatment of symptoms associated with the abnormal production of type I-IFN. These monoclonal antibodies are also appropriate for the preparation of diagnosis reagents.

A particular monoclonal antibody appropriate for the preparation of the pharmaceutical compositions for use as immunomodulator according to the present invention is directed against the human type I-interferon receptor (IFN-R) and is characterized by the following properties :

- it recognizes the extracellular domain of the human IFN-R, and
- it has a neutralizing capacity against the biological properties of the human type I-IFN.

The ability to neutralize the biological properties of type I-IFN can be estimated as a function of the capacity of the monoclonal antibody to neutralize the antiviral activity of the type I-IFN. Such a test is relevant in order to determine whether the antibody assayed is appropriate for the preparation of a pharmaceutical composition within the scope of the invention, although it is clear that the biological properties of type I-IFN are not limited to its antiviral properties. Detailed procedures are given in the examples in order to enable to perform such a test of the antiviral activity. The cells tested can advantageously be Daudi-cells, which affinity for the type I-IFN is well known. The main steps of such a test would consist in :

- incubating a determined concentration of human cells responsive to human type I-IFN, with human type I-IFN in the presence of a determined concentration of monoclonal antibodies to be assayed, for a time sufficient to allow the formation of a complex between the monoclonal antibodies and the IFN-R of the human cells and/or between the type I-IFN and the IFN-R of the human cells ;
- infecting the incubated cells with a determined virus, in a determined concentration,
- washing the cells,
- resuspending the cells in culture medium,
- incubating for a time sufficient to allow virus replication ;

- lysing the cells ;
- measuring the virus replication, or measuring the inhibition of the cytopathic effect.

The ability of the monoclonal antibodies of the invention to neutralize the biological properties of the human type I-IFN can be modulated as a function of the dose of antibodies used. Accordingly a 100% inhibition of the biological properties, or a partial inhibition can be obtained.

The following pages will refer to the definition of preferred monoclonal antibodies directed against the human type I-IFN receptor, which are appropriate when purified, to constitute an active principle of the pharmaceutical compositions according to the invention. The purification of the monoclonal antibodies is done by usual techniques and can be ascertain by protein determination of the content of endotoxins by the Limulus test.

According to a particular embodiment of the present invention, the monoclonal antibodies directed against the human type I-IFN receptor, are further characterized by the fact that they are capable of inhibiting the binding of a human type I-IFN, to the human IFN-R.

A monoclonal antibody having the capacity to recognize the extracellar domain of the human IFN-R and capable of inhibiting the binding of the human type I-IFN to its receptor, can be selected by the following steps :

- preincubating a determined concentration of purified monoclonal antibodies or a hybridoma culture supernatant containing monoclonal antibodies to be assayed, with human cells capable of harboring IFN-R ;

- adding labelled human type I-IFN, in a determined concentration, to the above preincubated medium ;
- incubating the medium containing the human cells, the monoclonal antibodies and the labelled type I-IFN for a time sufficient to allow an equilibrium to occur, between the monoclonal antibodies on the one hand and the type I-IFN on the other hand, with the cellular IFN-R ;
- washing the cells ;
- determining the formation of a binding complex between the human cells and the labelled type I-IFN by counting the amount of attached labelled type I-IFN.

Some of the monoclonal antibodies of the invention, have also the capacity to neutralize the antiproliferative properties of the human type I-IFN. This property can also be assayed on Daudi cells, by performing the following steps :

- allowing cells to grow in presence of human type IFN and determined concentration of mAb ;
- counting the cells in order to detect an inhibition of the antiproliferative effect of the human type I-IFN.

One property of a monocolonal antibody having an activity of immunomodulator, appropriate for the preparation of a pharmaceutical composition of the invention, resides in its capacity to recognize the extracellular domain of the human IFN receptor. This property of the monoclonal antibody can be assayed on human cells bearing the natural human receptor but also on the extracellular domain of a recombinant IFN-R such as expressed in a procaryotic cell, for instance in E.coli or a recombinant IFN-R such as expressed in a

eucaryotic cell such as mamalian cell for instance a CHO-cell.

This receptor can indeed present different properties, depending on the fact that it is produced in a procaryotic or eucaryotic cell and accordingly depending on the fact that the post-translational maturation occurred or not. The inventors interestingly showed that relevant assays, to evaluate the capacity of a monoclonal antibody according to the invention i.e. to recognize the cellular IFN-R, can be performed on a recombinant receptor expressed in mamalian cells. As a matter of fact, such recombinant receptor has the same properties as the cellular receptor, as far as its recognizing activity is concerned.

Monoclonal antibodies useful for the achievement of the invention can be obtained against various forms of the receptor, including the complete receptor, a particular domain or a peptide characteristic of the aminoacid sequence of the receptor represented in figure 3.

Monoclonal antibodies useful for the invention can for example be prepared against the soluble form of the receptor. A hydrosoluble polypeptide corresponding to the soluble form of the INF-R is described on figure 2. According to the present invention, a soluble form of the IFN-R corresponds to a peptide or a polypeptide, capable of circulating in the body.

Other monoclonal antibodies useful for the invention can also be prepared against a peptide comprised in the extracellular domain of the receptor as described on figure 2. An advantageous peptide corresponds for instance to the aminoacid sequence comprised between aminoacid 1 and aminoacid 427. According to another embodiment of the invention, the

antibodies can be prepared against a polypeptide modified by substitution of one or more amino acids, provided that antibodies directed against the non modified extracellular domain of the IFN-R, recognize the modified polypeptide or peptide.

Preferred monoclonal antibodies according to the invention are those which are of the IgG1 type.

Among the antibodies described hereabove, an antibody which has the capacity of inhibiting the binding of the type I-IFN to its receptor is preferably characterized in that it inhibits the in vitro binding of human type IFN, to the human cellular IFN-R when it is co-incubated with cells harboring the hu-IFN-R, at a concentration of antibodies equal or inferior to 100 µg/ml, preferably equal or inferior to 50 µg/ml, advantageously inferior to 20 µg/ml, more preferably in the range of approximately 0.5 to 2 µg/ml.

The inventors have shown that the high affinity binding capacity of a monoclonal antibody is not sufficient to ensure that this antibody will be able to inhibit the binding activity of the human type I-IFN to the IFN-R. Nevertheless the high affinity binding capacity of the monoclonal antibody is necessary to investigate further the ability of the antibody to inhibit the binding of the type I-IFN to its cellular receptor.

Another monoclonal antibody is characterized in that it neutralizes in vitro the antiproliferative activity of human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 10 µg/ml.

According to another embodiment a monoclonal antibody is also characterized in that it neutralizes in vitro the antiproliferative activity of human type

IFN, on cells poorly responsive to this human IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 µg/ml.

A particular group of monoclonal antibodies appropriate for the preparation of compositions for use as immunomodulators according to the invention, is characterized in that it neutralizes the antiviral activity of the human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 50 µg/ml, preferably 1 to 20 µg/ml, for a concentration of type I-IFN in the range of 1 to 1000 units with reference to the international standard MRC 69/19.

Advantageously, the monoclonal antibody according to the invention is such that these antibodies do not bind to the human receptor for IFN gamma.

One particular antibody interesting for the preparation of pharmaceutical compositions of the invention, is such as it directed against an epitope on the amino-acid sequence comprised between amino-acid 27 and amino-acid 427 of the extracellular domain of the human IFN-R as represented on figure 2.

One particularly interesting monoclonal antibody is the antibody designated 64G12 under n° 92022605 which has been deposited at the ECACC (European Collection of Animal Cell Cultures Porton Down Salisbury, Wiltshire SP4 056, United Kingdom) on February 26, 1992.

These antibodies may be prepared by conventional methods involving the preparation of hybridoma cells by the fusion of myeloma cells and spleen cells of an animal immunized beforehand with the peptide antigen, on the conditions such that the antigen against which the antibodies are formed is constituted by the

extracellular domain of IFN-R or any polypeptide or peptide of this domain.

The hybridomas are constructed according to the protocole of Kohler and Milstein (Nature, 1974, 256: 495-497). For example the hybridomas are derived from the fusion of the spleen cells above described with NS1 mouse (BalbC) HGPRT$^-$ as myeloma cell.

A second procedure for the production of monoclonal antibodies according to the invention, consists in carrying out the fusion between B-cells of blood immortalized with the Epstein/Barr virus and human B lymphocytes placed beforehand in contact with the extracellular domain or a fragment thereof of the IFN-R, against which it is decided to form monoclonal antibodies. B-cells placed in contact beforehand with the extracellular domain of IFN-R or fragment thereof against which it is decided to form monoclonal antibodies, may be obtained by in vitro culture contacted with the antigens, the recovery of the B-cells coated with these antigens being preceded by one or several cycles of stimulation.

The invention also concerns the use in the pharmaceutical compositions of human antibodies as obtained by carrying out the above procedure, having the above defined properties.

The invention also relates to the use in the pharmaceutical compositions of a monoclonal antibody characterized in that the variable or complementary determining regions of its heavy and/or light chains are grafted on the framework and/or constant regions of a human antibody.

A preferred pharmaceutical composition according to the invention is one which contains the purified preparation of monoclonal antibodies at a dose between

around 0.05 mg/kg of bodyweight and 3 mg/kg, preferably between 0.5 mg/kg and 1 mg/kg.

The pharmaceutical composition of the invention can be administered by different ways and especially the active principle can be combined with a pharmaceuticle vehicle appropriate for the intravenous or intramuscular administration.

According to a particular embodiment, the monoclonal antibodies which have been defined hereabove, can be used for a preparation of a drug having an immunomodulator effect sufficient to inhibit in vivo the disease due to the infection by a human retrovirus, especially by a human HIV or HTLV retrovirus.

Although several prior results already showed that interferon would be involved in the pathology of AIDS, this is the first time that the composition of the interferon present in case of infection by a HIV retrovirus is demonstrated, and this is the first time that it is demonstrated that monoclonal antibodies against type I-IFN receptor can be useful for the treatment of the diseases associated with AIDS, by acting on the immune response following the infection.

According to another example, such a drug can be used for the treatment of autoimmune and inflammatory diseases. Such diseases include systemic lupus erythematosus, type 1 diabetes, psoriasis, rheumatoid arthritis, multiple sclerosis, Behçet's disease, asplatic anemia, acquired immunodeficiency syndrome (AIDS), and severe combined immunodeficiency disease.

Thus the purified monoclonal antibodies defined above can be used for the preparation of a drug having an immunomodulator effect sufficient to inhibit in vivo the rejection of allografts or can be used for the

preparation of a drug having an immunomodulator effect sufficent to inhibit in vivo the symptoms of the Graft Versus Host Disease.

Treatment of symptoms of acute virus diseases can also be performed with the antibodies of the invention. As example upper respiratory tract infections, chronic virus infections such as those due to measles virus, can be performed.

The compositions of the invention can also be used for the in vitro diagnosis of the presence of the human soluble type I-IFN receptor or cells in a biological sample for instance in blood or in another fluid. The test can be made by several techniques such as ELISA (figure 14(2)) or RIA.

According to another aspect, the invention relates to a pharmaceutical composition for use as immunosuppressor, this composition being characterized in that it comprises as active principle, monoclonal antibodies having the above definition and as a combined preparation for simultaneous, separate or sequential use, an agent having an immunomodulator activity, especially an immunosuppressor activity such as cyclosporin A or FK 506. Especially the monoclonal antibodies are present in a dose ranging from 0.05 mg/kg to 3 mg/kg, preferably from 0.5 mg/kg to 1 mg/kg and the agent having immunosuppressor activity, especially cyclosporin A.

The results which are give hereafter show that the monoclonal antibodies which have been defined above can be used as adjuvant for humoral or cellular immunity.

Further details and additional information will arise from the description from the description of the examples and from the figures.

FIGURES

- Figure 1 : binding of ^{125}I-labelled monoclonal antibodies 34F10 and 64G12 to :
  - A : Daudi cells
  - B : Ly28 cells

  Briefly, 10^6 cells were incubated for 2 hours at 4°C in presence of different amounts of the labelled antibodies diluted in RPMI medium containing 10% fetal calf serum (FCS). The cells were then washed 4 times in RPMI-1% FCS and counted for bound radioactivity. Nonspecific binding was mesured by incubation with a 100 fold exces of cold antibodies and substracted from total counts.

- Figure 2 : nucleotide and corresponding amino-acid sequence of the extracellular domain of the human IFN-R

The monoclonal antibodies were produced against recombinant soluble forms of the human interferon alpha-beta receptor (IFN-R) synthetized in either procaryotic cells (E.coli) or a mammalian cell system (Cos cell). These soluble forms were based on the DNA sequence described in figure 2.

- Figure 3 : nucleotide and corresponding amino-acid sequence of the human IFN-R.

- Figures 4 to 28 : results relating to the immunomodulator activity of the antibodies.

E X A M P L E S

EXAMPLE 1 :

Synthesis of the soluble receptors
Synthesis in E.coli

A fragment of DNA containing the sequence coding for the extracellular domain (amino acids 27 to 427) of the human INF-R (figure 2), in which an extra-sequence coding for 5 histidyl residues was introduced just before the termination codon, was cloned in the expression vectors pKK233-2. This fragment was produced by the Polymerase Chain Reaction (PCR) and the resulting plasmids were sequenced to confirm both in-frame insertion with the Shine-Dalgarno sequence and the appropriate sequence coding for the receptor.

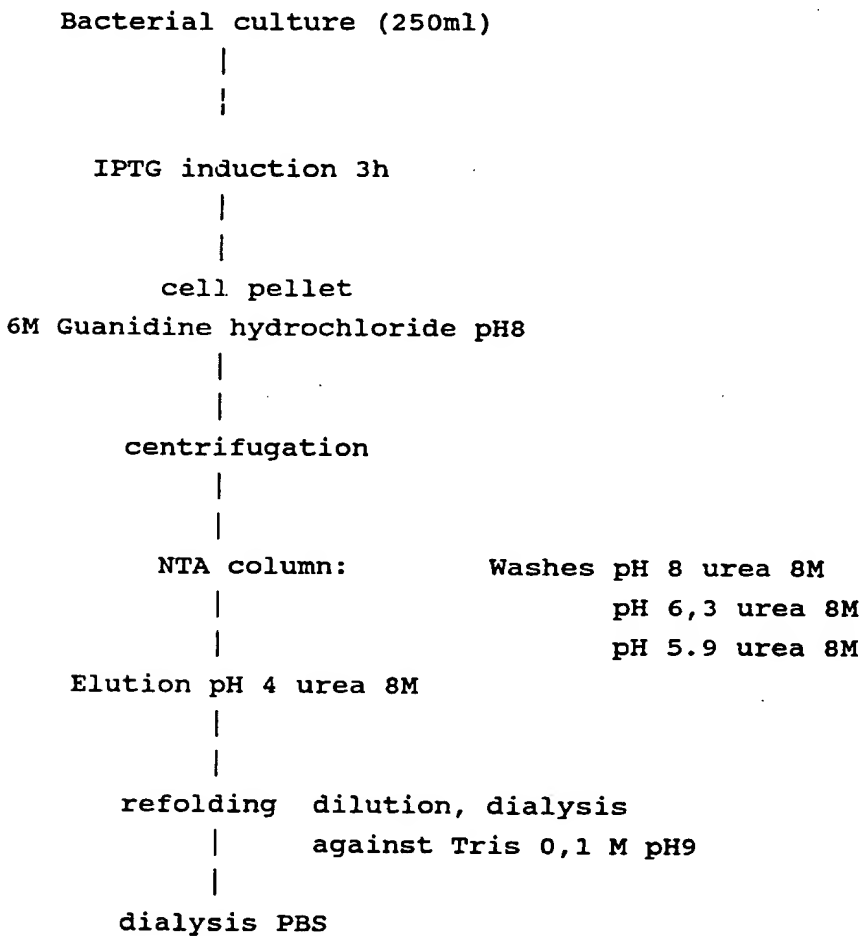
The poly-histidyl tail introduced into the recombinant protein enables it to be purified rapidly by affinity chromatography on a chelated nickel support (NTA column) as described previously (Hochuli E. et al, Bio/technology, 1988, 1321-1325).

The plasmid was introduced into the E.coli strain, JM105, and protein synthesis induced by addition of IPTG to the culture medium (pKK233-2, tac promoter).

Proteins were extracted from the bacterial pellet and the soluble receptor purified to homogeneity by affinity chromatography as described hereafter. This procedure yieled a protein that migrates as 2 bands around 50 kDa under reducing conditions and three bands under non-reducing conditions. The maximum concentration of the protein obtained by different procedures was approximately 20μg/ml.

The N-terminal sequence of the two proteins detected by gel electrophoresis has shown that both proteins are the expected fragment of the receptor.

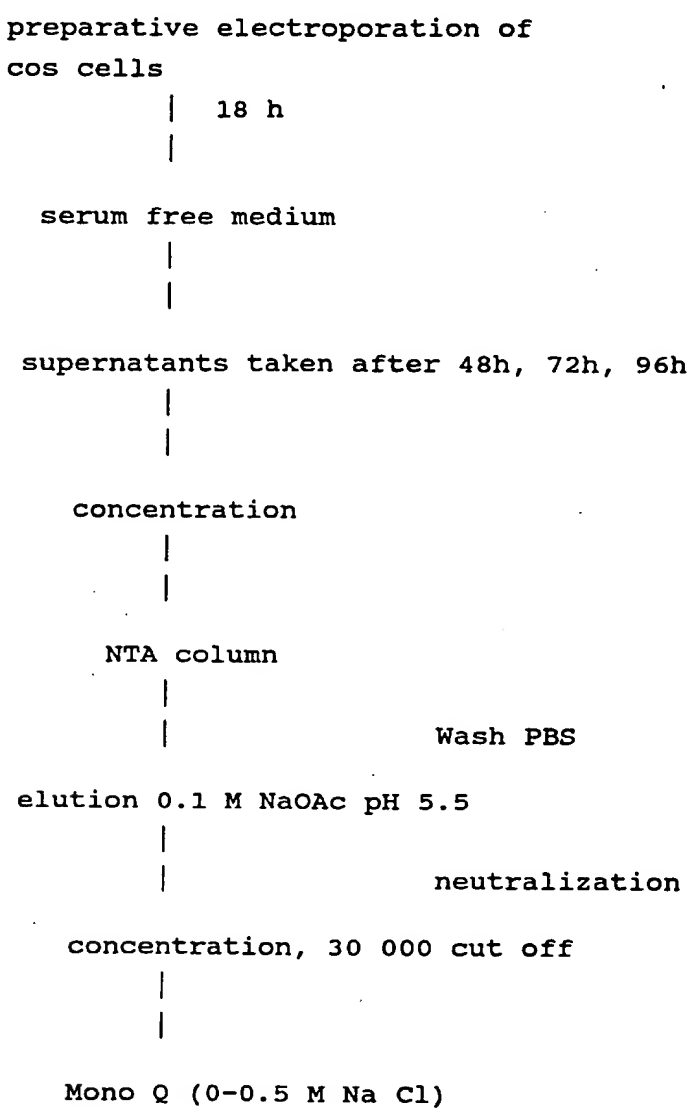
Synthesis and purification of an unglycosylated soluble receptor :



Using the same PCR approach, we also constructed an expression vector coding for the IFN-R amino acid sequence 1-427, with an additional 5-histidyl residues at the C-terminus, inserted in expression vector pXMT-3. The exact nucleotide sequence of the insert was also confirmed.

The resulting plasmid was introduced by electroporation into Cos7 cells for transient expression and the recombinant protein was purified to homogeneity by affinity chromotography followed by ion exchange chromatography on mono-Q (Pharmacia) as described hereafter.

Purification of the soluble IFN-R from Cos7 cells



This purification yielded to a 76 kDa protein whose N-terminal sequence corresponds to the predicted receptor sequence with some heterogeneity in the processing of the leader sequence.

EXAMPLE 2 :

Production of monoclonal antibodies against the interferon type I receptor

1) Production of the monoclonal antibodies

Mice were immunized by injection of recombinant soluble interferon (r sIFN-R) purified from E.coli or from a culture supernatant of Cos7 cells. Initially Balb/C mice were injected both intraperitoneally and subcutaneously with the purified protein in complete Freund's adjuvant. Subsequently mice were injected once a week intraperitoneally with the purified proteins diluted in buffered saline solution. Ten micrograms of recombinant proteins were injected each time.

After the fourth injection, blood was collected and the presence of specific serum antibodies were tested by both ELISA and Western blot against the recombinant receptor. The strongest responders were then boosted with a total of 10µg of antigen half of which was injected intravenously and half intraperitoneally.

2) Cell fusion

Four days after boosting, spleen cells from the immunized animal were collected and fused to NS1 (mouse) (Balbc) HGPRT$^-$ myeloma cells according to the method described by S. Fazekas et al. (J. Immunol. Methods 35:1-32, 1980). Briefly, $5x10^7$ spleen cells were fused to $3x10^7$ myeloma cells in 1ml of polyethylene glycol solution and distributed in five 96 well plates on a peritoneal macrophage feeder layer in HAT (hypoxanthine, aminoprotein and thymidine) medium. This procedure was repeated 4 times as $20x10^7$ spleen cells were obtained from the immunized mouse. Screening for specific hybridomas was undertaken when large colonies were detectable in culture wells.

For the screening, presence of specific antibodies was determined by a direct ELISA method :

a) ELISA plates were coated overnight at 4°C with purified E.coli-expressed or Cos7 cell-expressed sIFN-R diluted in PBS. Plates coated with BSA were used to detect non specific binding,

b) Plates were saturated by incubation with 3% BSA in PBS for 1 hour at 37°C,

c) Plates were incubated for 4 hours at room temperature with hybridoma supernatants diluted 1 in 4 with PBS-0.05% Tween 20,

d) Bound antibodies were detected by a two step procedure, comprising a first incubation with goat anti-mouse biotinylated immunoglobulin followed by streptavidin-horseradish peroxidase complex (both from Amersham and diluted 1/1000 in PBS-0.05% Tween 20).

Positive antibody secreting hybridomas were passaged in 24 well plates on a spleen cell feeder layer and their reactivity was again checked by ELISA, and Western-blot.

3) Identification of reactivity to the natural interferon type I receptor

The reactivity of the monoclonal antibodies (mAbs) recognizing the recombinant sIFN-R was tested against the natural class I receptor expressed at the surface of Daudi cells, by membrane immunofluorescence. Briefly, $5x10^5$ Daudi cells were incubated in 100µl of culture supernatant of chosen hybridomas for 30 min at 4°C. The cells were then washed 4 times in RPMI medium containing 1% BSA and further incubated with a diluted FITC labelled goat anti-mouse $F(ab')_2$ for 30 min at 4°C. The cells were finally analyzed by flow cytometry after washing. One of the 35 tested antibodies produced against the E.coli recombinant receptor and 5 of the 6 tested antibodies produced against the COS recombinant receptor were found to recognize the natural receptor on the Daudi cells.

Cloning of these hybridomas was then performed by limiting dilution. The isotype of these mAbs was determined by an ELISA method using isotype specific antibodies. All 6 mAbs were found to be IgG1 with kappa light chains. A summary of the reactivity of these 6 mAbs is given in Table 1.

Monoclonal antibodies were purified from culture supernatants by protein G chromatography.

Table 1 :

Reactivity of the anti IFN-R monoclonal antibodies

	Reactivity against the recombinant receptor				Reactivity against * the cellular receptor
	E.COLI		COS		
	ELISA	Western	ELISA	Western	immunofluorescence
34F10	+	+	+	+	+
64G12	+	+	+	+	+
63F6 64G2 64D10 65D8	-	-	+	+ weak	+

* measured on Daudi cells

EXAMPLE 3 :

Inhibition of the binding of interferon to human cell lines

Inhibition of interferon binding to human cells was assayed as follows. 10^6 cells were preincubated at 4°C for 30 min with various dilutions of hybridoma culture supernatants or purified mAbs or with medium alone. ^{125}I-labelled IFN alpha 8 or alpha 2 was added at the concentration of 100pM and cells incubated for a further 2 hours at 4°C. These incubations were performed in RPMI medium containing 20mM HEPES pH 7.4 and 10% foetal calf serum (FCS). The cells were finally washed 4 times with RPMI - 1% FCS and counted to determine bound radioactivity.

The mAb secreted by the hybridoma line 64G12 (latter named mAb 64G12) was shown in this assay to inhibit the binding of labelled IFN to the cells in a dose-dependent manner. 50% inhibition of binding to the Daudi cells (Burkitt lymphoma cell line ; Klein et al., Cancer Researh, 28:1300-1310, 1968) was obtained at a mAb concentration of 0.4µg/ml. The same inhibition was obtained with K562 cells (chronic myelogenous leukemia, Lozzio and Lozzio, Cell, 45:321-334, 1975) but 50% inhibition was obtained at 11µg/ml for HL60 cells (Promyelocytic leukemia, Collins S.J. et al., Nature, 270:347-349, 1977) and 60µg/ml for Ly28 cells (Klein G. et al. Int. J. Cancer, 10:44-57, 1972).

Table 2 :

The inhibition of binding of labelled IFN alpha 2 to various cell lines by mAB64G12

Cell lines	Concentration of mAB which gives 50% inhibition of binding
Daudi K562	0,4 µg/ml
HL60	11 µg/ml
Ly28	60 µg/ml

The difference in the mAb concentration at which 50% inhibition of binding of IFN is obtained has been investigated by direct binding of ^{125}I-labelled mABs 64G12 and 34F10 to the same cell lines and Scatchard

plot analysis of the results. In the concentration range of 0.1 to 1.5 µg/ml, a high affinity binding of the mAb 34F10 (≈10nM) was seen on all cell lines whereas a high affinity binding of mAB 64G12 was only detected on Daudi and K562 cells (Figure 1).

EXAMPLE 4 :

Inhibition of the function of type I interferon

Functional inhibition of type I interferon by the purified mAb 64G12 was firstly demonstrated in an antiviral assay on Daudi cells using either recombinant IFN alpha 2, IFN beta and IFN omega, or purified Namalwa and leucocyte interferons, and in an antiproliferative assay with recombinant IFN alpha 2.

* Antiviral activity

An antiviral assay on Daudi cells was performed as described (M. Dron and M.G. Tovey, J. Gen. Virol. 64:2641-2647, 1983). Cells ($0.5x10^6$/ml) were incubated for 24 hours in the presence of interferon and antibodies. 10^6 cells in 1 ml were then infected for 1 hour at 37°C with Vesicular stomatitis virus (VSV) then washed 3 times, resuspended in culture medium and incubated for 18 hours at 37°C. Cells were then lysed by freeze-thawing and virus replication measured by titration of the supernatants on L929 cells. A dose-dependent inhibition of the antiviral activity of the various subtypes of type I IFN was demonstrated for the purified mAb 64G12.

For the antiviral assay with the Wish cells, cells were incubated for 24 hours with various concentrations of interferons in the presence of the mAbs prior to challenge with VSV. In this assay, the mAb 64G12 was demonstrated to block completely the antiviral activity of Leukocyte IFN (50U/ml), recombinant IFN alpha 2 (50U/ml) and interferon from the sera of AIDS patients (50, 75 and 150U/ml).

* antiproliferative activity

For the antiproliferative assay, Daudi cells were seeded at a concentration of 10^5 cells per ml in a 96 well plate in the presence of interferon and purified inhibitory or control antibody. Cells were then counted after 24, 48 and 72 hours with a Coulter counter and checked for viability by trypan blue exclusion. Purified mAb 64G12 demonstrated a dose-dependent inhibition of the antiproliferative activity of interferon alpha 2.

EXAMPLE 5 : Inteferon and the pathogenesis of AIDS

I) The Role of Interferon α in the Pathogenesis of AIDS

A number of studies suggest that co-factors, including cytokines, play a determining role in the development of AIDS (Kramer et al., 1992, Lancet, 340:371; Vadhan et al., 1986, Cancer Res., 46:417), particularly in the suppression of the immune system, following infection with the Human Immunodeficiency Virus (HIV). Among the cytokines induced by HIV are the interferons which are thought to play an important role in host defense against viral infections. Although

inteferons can restrict the replication of HIV in vitro (Poli et al., 1989, Science, 244:575; Von Sydow et al., 1991, AIDS Res. and Human Retroviruses, 7:437), and recombinant interferon α2 has been shown to exert an antiviral and antitumoral effect in AIDS patients with Kaposi sarcoma (Krown et al., 1991, J. Acquired Immune Deficiency Syndromes, 4:671), the production of inteferon does not prevent the development of AIDS in HIV infected individuals. Furthermore, the restriction of HIV replication by inteferon α may contribute to the development of a latent infection in macrophages (Gendelman et al., 1990, J. Exp. Pathol., 5:53; Gendelman et al., 1990, AIDS Res. and Human Retroviruses, 6:1045; Mace and Gazzolo, 1991, Res. Virol., 142:2). An unusual acido-labile α inteferon has been shown to correlate with loss of CD_4+ cells and disease progression in these patients (Milvan et al. 1992, The Lancet, 339:453). α inteferons are potent immunoregulatory molecules which enhance MHC class I antigen expression, MHC restricted T-cell killing, and non MHC restricted NK cell killing, of virus-infected cells all of which could contribute to the loss of CD_4+ T-lymphocytes observed in HIV infected individuals. Thus, a marked reduction in the number of circulating CD_4+ T-lymphocytes has been observed in HIV infected individuals treated with recombinant interferon α2 (Vento et al., 1993, The Lancet, 341:958).

II) Characterization of the Endogenous Interferon Present in the Sera of AIDS Patients

In order to characterize the endogenous interferon present in the sera of AIDS patients peripheral blood leukocytes were isolated from HIV infected individuals with high levels of circulating interferon, RNA was extracted, reverse transcribed with MLV reverse transcriptase and a forward primer (a 20 mer synthetic oligonucleotide) specific for either a single gene (Tables 3-5, Figure 4) using standard procedures. The resulting cDNA was then amplified by the polymerase chain reaction (PCR) using one of the three pairs of specific oligonucleotide primers (Table 5) for a total of 30 cycles using standard procedures. The product was then identified either by Southern hybridization as in the case of the IFN β and α genes or by direct di-deoxy sequencing using T7 DNA polymerase (Sequenase, USB) as in the case of the α inteferons following a further 15 cycle of asymetric amplification (using 0.5 pmol of one of the pair of specific oligonucleotide primers and 50 pmol of the other pair of primers) using standard procedures. The results of this analysis showed that IFN α5 was the majority species expressed in approximately 30 samples of peripheral blood cells from HIV infected individuals whereas IFN αn (mainly IFN α1 and IFN α2) were the main interferon species expressed in peripheral blood cells from 5 HIV-seronegative individuals infected with rhinoviruses, influenza virus, or cytomegalovirus (Figure 5).

Similar results were also obtained when the expression of interferon genes in the promonocytic cell line U937 chronically infected or not with HIV-1 was analyzed using the same techniques. IFN α5 was found to be the main species expressed in cells chronically

infected with HIV-1 whereas IFN α1 and IFN α2 were the most abundent species expressed in uninfected U937 cells induced with either Newcastle Disease Virus (NDV) or the polyribonucleotide PolyI-Poly C.

These results may explain why monoclonal antibodies which neutralized IFN α2 did not neutralize the interferon present in the sera of AIDS patients (Tables 6, 7). Serum interferon from AIDS patients was not neutralized either by monoclonal antibodies specific for IFN β or IFN x but was neutralized, however, by a polyclonal antibody raised against human lymphoblastoid inteferon (Tables 6, 7).

For various reasons polyclonal antibodies cannot be used for the therapeutical treatment of patients infected with HIV.

III) Determination of the Capacity of a Monoclonal Anti-Interferon α Receptor Antibody to Neutralize the Biologic Activity of the Interferon Present in the Sera of AIDS Patients.

Pretreatment of WISH cells (a human amnion cell line) with the anti-interferon receptor antibody 64G12 at a concentration of 50μg/ml completely neutralized the antiviral activity of the endogenous interferon present in the serum of AIDS patients (Table 8). Pretreatment of WISH cells with the non-neutralizing antibody 34F10 at the same concentration had no effect on the antiviral activity of the endogenous interferon from AIDS patients (Table 8).

IV) Establishment of an animal model to test the effect of an inteferon antagonist on the evolution of the acquired immunodeficiency disease.

In order to determine whether IFN α plays a role in the pathogenesis of AIDS and to test the efficacity of interferon antagonists as therapeutic agents in this disease we have developed an animal model based on the use of rhesus macaques infected with the simian immunodeficiency virus (SIV). SIV is a CD_4+ tropic lentivirus which shares considerable nucleotide sequence homology, antigenicity, and biologic properties with HIV. SIV-induced disease in macaques is characterized by lymphadenopathy, immune deficiency, and opportunistic infections and is considered to be an excellent experimental model of human AIDS (McClure et al., 1990, AIDS : Anti-HIV Agents, Therapies and Vaccines. Annals NY Academy of Sciences, 616:287).

Primary Infection

Infection of macaques with SIV is followed by a peak of serum IFN α at 7 to 14 days post infection which falls to undetectable levels by 21 days (Figure 6). The peak of serum IFN α occurs 2 to 3 days prior to, or concomitantly with the peak of p27 antigenemia at 14 to 15 days post-infection (Figure 7). Both the peak of interferon α production and p27 antigenemia occur prior to the development of antibody to viral antigens which first becomes detectable at 21 to 25 days post infection (Figure 8). The production of IFN α in SIV-infected macaques occurs in the absence of detectable levels of IFN γ, IL-6, or TNF-α.

Late-Stage Disease

A group of 12 rhesus macaques infected with SIV and 9 uninfected control animals were followed for a period of some 30 months after infection. No interferon was detected in the sera of any of the uninfected

control animals at any time during the 30-month observation period. The development of AIDS-like disease in SIV-infected macaques was found to be preceded by a second peak of serum IFN α which occurs concomitantly with the loss of CD_4+ cells several months after primary infection. This second peak of interferon production, unlike that which is observed during primary infection, occurs over a period of several months, and precedes, the p27 antigenemia which occurs in late stage disease, in some cases by several months. Three types of interferon response are seen in late stage disease; the production of a peak of interferon α some six to nine months post infection just prior to the development of the clinical signs of disease and death (Table 9, animals 483, 485, and 495, Figures 9-11), the production of low levels of interferon α first detectable shortly after infection and which persist for up to 30 months (Table 9, animals 501, and 505, Figures 12-13), and the absence of detectable levels of interferon α during the whole 30 month observation period following infection with SIV (Table 9, animals 475, 456, 457, 489, and 489).

Inteferon α production was only observed in those animals which exhibited a weak or absence cytotoxic T-cell response (CTL) against autologous B-cells immortalized with Herpes papio virus and transfected with one of eight viral proteins (ENV, GAG, POL, NEF, VIF, REV, TAT, AND VPX) as described previously (Venet et al. 1992, J. Immunol., 148:2899). None of the animals studied which exhibited a strong CTL response had detectable levels of circulating inteferon at any time during the two year period of observation. IFN α was not detected in the serum of SIV-infected macaques with normal CD_4+ counts and without signs of disease.

Determination fo the Interferon Response in Chimpanzees Infected with HIV-1

Infection of chimpanzees with HIV does not lead to the development of AIDS-like disease eventhough the virus is able to replicate in these animals. The interferon response of a group of 5 chimpanzees was followed for several months prior to and for up to 20 months after infection with HIV-1 (Table 10). No inteferon α was detected in the serum of any of the 5 animals at any time either before or after infection with HIV-1 (Table 10). No decrease in the number of CD_4+ T-lymphocytes or any other sign of clinical disease was observed in any of the animals studied.

Conclusions

A close relationship has been observed between the presence of circulating interferon α in the serum of SIV-infected macaques, and a weak or absent CTL response, the decrease in CD_4+ cells, and the development of the clinical symptoms of immunodeficiency disease in these animals. Thus, this system appears to resemble the situation seen in man and would appear to represent a good model in which to test the potential efficacy of an interferon antagonist for the treatment of AIDS.

V) Toxicology and Pharmacokinetic Studies of The Anti-Inteferon α receptor Monoclonal 64G12 in Rhesus Macaques

Toxicology

The 64G12 antibody produced in vitro and purified by affinity chromatography on protein G sepharose,

after ultracentrifugation (100 000 x g), and filtration on a Millipore membrane (0.2μ), was found to be sterile and to be devoid of detectable endotoxin (Limulus test).

No toxicity was detected in normal adult mice either immediately, or up to three months after, the intravenous injection of 640 μg of a preparation of the 64G12 antibody. Similarly, no immediate or longterm toxicity was observed when new-born mice were injected intrathecally with the same preparation of antibody.

The 64G12 antibody was well tolerated in rhesus macaques following intravenous administration at a dose of 0.5 to 1.0 mg/kg. No local reaction was observed at the site of injection. Furthermore no systemic reactions such as fever, oedema, etc. were observed in any of the animals injected with the antibody. Successive injections of the antibody were also well tolerated. The only reaction observed was a slight facial oedema seen in certain animals immediately after the third intravenous injection at 15 days (previous injections were at 0, and 5 days). When the antibody was administered by intramuscular injection, at the third and subsequent injections no oedoma was observed.

An accident (anaphylactic shock) occured in a single monkey 20 minutes after the intravenous injection of a non-purified preparation of the 64G12 antibody at day following 4 previous intravenous injections of the antibody at days 0, 5, 10 and 15.

Pharmacokinetics and Establishment of the Lowest Effective Dose of the Monoclonal Antibody 64G12

The level of the 64G12 antibody present in the serum of animals following intravenous injection was determined using an ELISA test based on the use of recombinant soluble receptor to capture antibodies which recognize the extracellular domain of the human interferon α receptor (Figure 14).

ELISA for the Detection of Anti-interferon Type I receptor antibody

Briefly, ELISA plates were coated with a recombinant protein corresponding to the extracellular domain (amino acids 1 to 427) of the human interferon α receptor produced in either COS or CHO cells at a maximum concentration of 10 to 20.0 μg/ml in either PBS or 100 mM carbonate buffer, pH 9.2. The plates were then saturated with a 3% solution of bovine serum albumin or similar agent in PBS. The plates were washed with PBS containing 0.5 to 1.0% Tween 20 or a similar detergent and serial dilutions of the samples to be tested, or reference preparation, were then applied to the plates. The plates were then incubated overnight at 4°C or for 2 hours at 37°C, washed with PBS/Tween, and incubated for approximately 2 hours at 37°C with a polyclonal anti-mouse IgG conjugated with alkaline phosphatase, or horse-radish peroxidase. Biotin-streptavidin reagents can also be used (Sheep anti-mouse Ig and Streptavidin-biotinylated peroxidase performed complex from Amersham are suitable). The plates were then washed with PBS/Tween, incubated with the corresponding substrate (o-phenylene-diamine at a concentration of 0.4 mg/ml in a citrate buffer pH 5.5 is suitable for peroxidase; the reaction can then be stopped usually after 1 to 10 minutes by the addition of 0.5 M H_2SO_4) and the optical density determined (at

405 nm for peroxidase reactions) according to standard procedures.

The use of this ELISA for the determination of the concentration of the 64G12 in the sera of rhesus macaques showed that the pharmacokinetics of this antibody following intravenous injection were similar to those previously described for other mouse IgG1 monoclonal antibodies (Figure 15).

Determination of the Number and Affinity of the Interferon α receptors on the Peripheral Blood Cells of Rhesus Macaques

Binding studies using standard procedures (Mogensen, Uzé et al 1986, Methods Enzymol. 119:267) showed that the binding of ^{125}I labelled recombinant human IFN α2 to the peripheral blood mononuclear cells of normal untreated rhesus macaques was similar to that observed with human peripheral blood cells (Figure 16). Scatchard analysis (Figure 17) showed that macaque peripheral blood mononuclear cells exhibited approximately 1500 to 3500 receptors per cells with a kD ranging from 2 to 8 x 10^{10} M (Figure 18). The intravenous injection of 1.0 mg/kg of the 64G12 anti-interferon receptor resulted in serum levels of approximately 60 µg/ml, 30 minutes after administration of the antibody which is in good keeping with the expected levels taking into account the blood volume of the animals.

Treatment of macaques with the 64G12 anti interferon receptor antibody at a dose of 0.5 mg/kg was found to completely inhibit the binding of radiolabelled human interferon α 2 to the peripheral blood mononuclear cells of these animals for a period

of up to 5 days (Figure 19). Some 60 to 70% of the interferon receptors on the peripheral blood cells of treated animals became accessible again to the binding of radiolabelled IFN α2 by day 6 (Figure 19). A dose of 0.5 mg/kg was used as the minimal effective dose in all subsequent experiments.

VI) Determination of the Effect of the Anti-interferon receptor Antibody 64G12 on the Early Stages of Disease in SIV Infected Rhesus Macaques

A group of 8 rhesus macaques were infected with 10 macaque infectious dose $_{50}$ of a molecular clone of SIV strain 251. Two macaques were injected intravenously with 0.5 mg/kg of the anti-interferon receptor antibody 64G12, 30 minutes prior to infection with SIV, and at days 5, 10 and 15 post infection. One macaque was injected intravenously with 3.5 mg of a bull polyclonal anti-lymphoblastoid interferon IgG preparation, 30 minutes prior to infection and at days 5 post infection, and intramuscularly with 3.5 mg of the antibody at days 10 and 15 post infection. The other 5 infected animals were left untreated.

No significant difference in the peak level of p27 antigenemia which occurs at day 14 post infection was observed in any of the treated animals relative to the untreated control animals (Figure 20). The peak of interferon α production which occurs at dat 10 post infection was considerably higher in the animal treated with the polyclonal anti-interferon antibody, relative to that observed in the untreated control SIV-infected animals (Figure 21). In contrast, treatment of SIV-infected macaques with the monoclonal anti-interferon receptor antibody had non significant effect on

interferon production in these animals (Figure 21). Anti-p27 antibodies also appeared earlier and in larger amounts in the animal treated with the polyclonal anti-interferon antibody than in either animals treated with the 64G12 antibody or in untreated SIV-infected control animals (Figure 22).

Infection of macaques with SIV was followed by a marked decrease in the percentage of CD_4+ T-lymphocytes at 14 to 15 days post infection. In the two animals treated with the anti-interferon α receptor antibody the percentage of CD_4+ cells was found to decrease in a manner similar to untreated control animals and then to recover rapidly to attain in both animals higher levels than in either the animal treated with the polyclonal anti-interferon antibody or in untreated control SIV-infected animals (Figure 23). The difference in CD_4+ cells in the animals treated with the anti-interfeeron α receptor antibody persisted for approximately three weeks prior to decreasing slowly in parallel with that of the control animals. No significant effect on the level of CD_8+ cells was observed in any of the treated animals relative to the levels seen in untreated control animals. The polyclonal anti-interferon antibody had no significant effect on either the number or percentage of CD_4+ or CD_8+ cells.

EXAMPLE 6 : <u>The Effect of the Anti-Interferon α Receptor Monoclonal Antibody on Skin Allograft Survival in Cynomologus Monkeys.</u>

It is considered that cytokines produced both by sensitized T-cells and non MHC restricted cells play an important role in the processes which lead to allograft rejection. Numerous observations suggest that

interferon α produced during the initial stages of recognition plays a determining role in the initiation of the processes which lead to graft rejection. For example, treatment of lethaly irradiated mice with interferon α has also been shown to enhance resistance to allogenic bone marrow grafts while treatment with antibody to interferon α was found to inhibit rejection (Affifi et al. 1985, J. Immunol., 134:3739). Treatment of rats with antibody to inteferon α/β has also been reported to prolong cardiac allograft survival in these animals either when administered alone or together with cyclosporin A (Gugenheim et al., 1992, Transplant. Int. 5:460). The increased survival of cardiac allografts in animals treated with anti-interferon α antibody was associated with a decreased expression of MHC class I antigens on the grafted tissue (Gugenheim et al., 1992, Transplant. Int. 5:460). These results are in keeping with the fact that inteferon α is the principal activator of MHC class I antigens and NK-cell activity both of which play a major role in allograft rejection.

Numerous observations suggest that interferon α also plays an important role in the development of graft-versus-host disease (GVHD). Thus, interferon α is produced during the course of graft-versus-host disease in parallel with the enhanced NK cell activity characteristic of systemic GVHD and administration of interferon α has been shown to enhance the intestinal consequences of GVHD in normal mice (Cleveland et al., 1987, Cell Immunol. 110:120).

I) Effect of the Anti-Interferon α Receptor Antibody on the Allogenic Response of Mixed Lymphocyte Cultures.

In order to test the potential immunosuppressive activity of the anti-interferon α receptor antibody 64G12, mixed lymphocyte cultures (MLC) were treated with varying concentration of either the 64G12 antibody, or the non-neutralizing control antibody 34F10. Both antibodies had been shown previously to be without effect, on either the viability or proliferation of either human peripheral blood mononuclear cells or the human lymphoblastoid cell line Daudi after 96 hours incubation in vitro at a concentration of 50 μg/ml.

Human peripheral blood mononuclear cells from normal donors were isolated on a Ficoll gradient and typed for MHC class I and class II antigens by complement dependent micro-lymphocytoxicity. The proliferation of the responder cells was determined by measuring the incorporation of ^{3}HTdR in response to allogenic stimulatory cells rendered non-proliferative by treatment with mitomycin-C. Treatment of mixed lymphocyte cultures with the anti-interferon α receptor antibody resulted in a dose dependent inhibited the incorporation of ^{3}HTdR (Figure 24). A mean inhibition of approximately 50% in the incorporation of ^{3}HTdR was observed in MLC treated with the 64G12 antibody at a concentration of 20 μg/ml of (Figure 24). The degree of inhibition of ^{3}HTdR incorporation varied considerably, however, from one MLC to another (20 to 82% with a mean of 50.58 + 20.01% for a series of 12 MLC, Figure 25) without any clear relation to the MHC DR type of either the donor or responder lymphocytes (Table 11). No inhibition of ^{3}HTdR incorporation was observed in cultures treated with up to 50μg/ml of the non-neutralizing anti-interferon α receptor antibody 34F10.

II) Effect of the Anti-interferon α Receptor Antibody on the Survival of Skin Allografts in Cynomologus Monkeys.

Skin allografts were exchanged between AOB compatible 1 to 3 year old male animals (Macaoa Fascicularis) differing in both MHC class I (Rh LA-A and B) and class II antigens (Th LA-DR) using standard procedures. The grafts were taken and implanted in the left illiac fosse. Animals were either left untreated, or treated (intramuscular injection) with cyclosporin A (5.0 mg/kg/day) one hour prior to grafting and each subsequent day, or with the 64G12 antibody (0.5 mg/kg) one hour prior to grafting and on day 5 and every subsequent 5th day (by intravenous injection for the first two injections and then by intramuscular injection subsequently) until day 85 or until rejection of the graft, or treated with cyclosporin A together with the 64G12 antibody at the same doses as used for each substance alone.

The grafts were examined daily for evidence of the clinical signs of rejection (colour, suppleness, etc.) and grafted tissue was biopsied at days 5, 10, 20, and 60 and at the day of rejection. Each biopsy was examined for histological signs of rejection and for the expression of MHC class I and Class II antigens.

The mean survival time of skin grafts in the untreated control animals was found to be 7.5 + 0.57 days, and 9.5 + 0.57 days in cyclosporin A treated animals (Table 12). Treatment of animals with the 64G12 antibody was found to increase significantly the mean survival time of the grafted tissue (14.25 ± 0.95 days) relative to both the untreated control animals (Wilcox

test. $p<0.01$) and animals treated with cyclosporin A (Table 12).

Treatment of animals with the anti-interferon α receptor antibody together with cyclosporin A resulted in permanent graft acceptance (Table 12). Indeed graft rejection was observed at 63 days in a single animal only while the grafts in the remaining 4 animals in this treatment group remain fully viable and without any signs of rejection at 235 days (Table 12). Treatment with the anti-interferon receptor antibody was stopped at day 85 in those animals with intact skin-grafts. These animals continued, however, to be treated with low dose cyclosporin A (5.0 mg/kg) until day 140 at which time all treatment was stopped.

A single monkey in this group was lost at day 20 due to anaphylactic shock 20 minutes after the intravenous injection of an unpurified preparation of the 64G12 antibody. The skin graft in this animal showed no macroscopic or microscopic signs or rejection at this time.

Histologic examination of biopsies of surviving skin-grafts, taken at 60 days, from animals treated with anti-interferon receptor antibody together with cyclosporin A showed only rare mononuclear cells under the epidermis and around certain capillaries. Skin biopsies taken at 185 days from animals which had been treated with anti-interferon receptor antibody and cyclosporin A for 85 and 140 days respectively revealed tissue which was difficult to distinguish from normal ungrafted skin. A moderate inflammatory infiltrate consisting essentially of interstitiel and or percapillary mononuclear cells was seen in biopsies

taken from animals treated. This contrasted with the very severe lesions (necrosis oedema, marked inflammatory infiltrate with numerous polynuclear neutrophiles) seen in biopsies taken from the untreated control animals. Biopsies taken from animals treated with cyclosporin A alone exhibited a dermic and hypodermic inflammatory infiltrate consisting essentially of mononuclear cells and some polynuclear neutrophiles with moderate oedema.

The skin allograft biopsies were also examined for the expression of MHC class I (HLA-ABC) and class II antigens (HLA-DR). The biopsies from the untreated control animals exhibited a marked expression of both class I and class II antigens (Table 13). The expression of MHC class I antigens in the biopsies taken from animals treated with cyclosporin A was similar to that of untreated control animals while the expression of class II antigens was less intense (Table 13) whereas in biopsies taken from animals treated with the anti-interferon receptor antibody the expression of MHC class I antigens was reduced while that of class II antigens remained unchanged relative to control animals (Table 13). In contrast, in biopsies taken from animals treated with the 64G12 antibody together with cyclosporin A, both MHC class I and class II antigens were markedly reduced relative to untreated control animals (Table 13).

A hyperlymphocytosis was observed in animals treated with either the anti-interferon receptor antibody alone or together with cyclosporin A (Table 14). This hyperlymphocytosis was associate with an increase in the percentage of CD8+ T-lymphocytes relative to untreated control animals. No difference in

either the absolute number or percentage of CD4+ T-lymphocytes was observed in animals treated with the anti-interferon receptor antibody relative to control animals (Table 15). No differences were observed in the erythroid or platelet cell lineages.

No differences in the levels of blood sodium or potassium creatin were observed in any of the animals following transplantation (Table 16).

Monkeys treated with cyclosporin A (5.0 mg/kg/day) together with the anti-interferon α receptor antibody exhibited significantly higher levels of serum cyclosporin A than animals treated with the same dose of cyclosporin A alone (Table 17).

No detectable monkey antibodies (IgG) to the mouse anti-human interferon α receptor antibody were detected, using a sensitive ELISA specific for anti-interferon α receptor antibodies (Figures 26-28), in the serum of any of the animals treated with 0.5 mg/kg of the antibody every 5 days (for up to 60 days) either alone or together with cyclosporin A (Figure 28). The apparent absence of immunogenicity of the 64G12 monoclonal antibody may be a consequence of the very strong immunosuppression observed in animals treated with this antibody.

Six months after the initial skin graft and two months after all treatment had been stopped animals were given a second skin graft from the same donor without further treatment. The secondary grafts were rejected at 5.5 ± 0.5 days in the untreated control group and at 11.5 ± 2.0 days in animals which had been treated initially with anti-interferon α receptor antibody together with cyclosporin A (Table 18). In

contrast, the primary skin grafts remained intact in those animals which had been treated intially with anti-interferon α receptor antibody together with cyclosporin A even though secondary skin grafts were rejected in these animals. These results suggest that the local production of interferon α within and or at the site of the grafted tissue plays a determining role in the initial processes which lead to allograft rejection.

These results show that treatment of cynomologus monkeys with the 64G12 antibody markedly increases allograft survival in these animals. The amplitude of the effect observed with the antibody alone is such that the 64G12 may be considered to be among the most potent immunosuppressive monoclonal antibodies described to date. Thus, monoclonal antibodies against interferon γ or TNF α appeared to be less effective in preventing skin allograft rejection in monkeys than the 64G12 antibody (Stevens et al., 1990, Transplantation, 50:856), while an anti-CD3 antibody (Nooji et al., 1987, Eur. J. Immunol. 17:1089) is the only antibody to give an effect comparable to that of the anti-interferon α receptor antibody 64G12.

Treatment of cynomologus monkeys with anti-interferon α receptor antibody together with cyclosporin A resulted in permanent allograft acceptance in non MHC matched animals which contrasts markedly with the limited efficacy of other immunosuppressive agents in the primate model.

The very strong synergic immunosuppressive effect observed in animals treated with the anti-interferon α receptor antibody administered together with low dose

cyclosporin A would enable effective immunosuppression to be attained at doses of cyclosporin A which are normally ineffective thus reducing the severe toxicity observed in patients treated over long periods of time with high doses of cyclosporin A. Furthermore, the fact that allograft survival is maintained after treatment with the anti-interferon α receptor antibody has been stopped suggests that only a limited period of treatment with the 64G12 antibody may be necessary to attain effective immunosuppression.

It is probable that given their similar mode of action comparable immunosuppressive effects would be observed if the 64G12 antibody was administered together with FK 506.

These results, which show that selective and lasting immunosuppression can be obtained by the administration of relatively low levels of an interferon antagonist given together with low dose cyclosporin A, may have important implications for the prophylaxis and treatment of human allograft rejection.

Such treatment regimens may also be applicable to the treatment of autoimmune disease particularly those diseases characterized by the abnormal, or prolonged production of interferon α.

III) Effect of Anti-interferon α Receptor Antibody on graft-versus-host disease in cynomologus monkeys.

Primates provide a near optimal model for preclinical investigation of bone marrow transplantation in that their radiosensitivity, the composition of their bone marrow, and the pattern of

their immunological reactions to bone marrow grafting are closely similar to those of humans. Furthermore, modifications of the acute graft-versus-host-disease (GVHD), which develops in the monkey after grafting marrow from unrelated non MHC matched donors, have proven to be highly predictive of the human situation (van Bekkam, Transplantation Proceedings, 10, 105-111, 1988).

Young adult male animals (Macaca Fascicularis) were treated with broad spectrum antibiotics to attain intestinal sterilization prior to irradiation.

Animals were irradiated with a whole body dose of 8 Gy and grafted 4 hours later with 4 x 10^8 allogeneic bone marrow cells/kg from random donors differing in both MHC class I (Rh LA-A and B) and class II antigens (Th LA-DR).

Animals were either left untreated, or treated with anti-interferon α receptor antibody 30 minutes prior to grafting and on day 5 and every subsequent 5th day (by intravenous injection for the first two injections and by intramuscular injection thereafter) together with low dose cyclosporin A (5.0 mg/kg) 30 minutes after grafting and daily thereafter.

Surviving animals were housed in isolation rooms for the first month following irradiation and under open conditions thereafter.

Untreated grafted animals died at 9 ± 1.0 days with an acute form of GVHD with characteristic histologic lesions of the skin similar to those seen in man (Table 19). Of the three grafted animals treated to date with the anti-interferon α receptor antibody

together with low dose cyclosporin A one animal died from a bacterial infection at 19 days without clinical or histological signs of GVHD. While the two other animals remain healthy at 93 days post transplantation (Table 19).

These preliminary results suggest that anti-interferon α receptor antibody given together with low dose cyclosporin A may be an effective treatment for GVHD. Very few agents are effective in mitigating the acute from of GVHD seen in primates and in prolonging survival in these animals. Indeed, a beneficial effect in this model is considered to be predictive of a correspondingly greater beneficial effect in the less severe type of GVHD seen in patients receiving bone marrow from MHC matched donors.

GENE	Nucleotide Position	Primer	Product Size	Sequence 5' ——→ 3'
IFN α	982-1005	Forward		GATCTCATGATTTTCTGCTCTGACA
	723-743	Reverse	283 bp	TCCATGAGATGATCCAGCAGA
	902-924	Probe		ATCCTGGCTGTGAGGAAATACTT
IFN ω	491-512	Forward N.1		ATGTTCCAGGCAGCAGAGGAGC
	438-457	Forward N.2	305 bp	GCATCTCATGGAGGACAGAC
	208-230	Reverse N.1	(1st round)	ATGGCCCTCCTGTTCCCTC
	257-276	Reverse N.2	202 bp	GCCCTGTTGGATCTCTGGGC
	359-387	Probe	(2nd round)	TGTGTCTCAAGGACAGAAGAGACTTCAGG

TABLE 3

GENE	Nucleotide Position	Primer	Product Size	Sequence 5' ——→ 3'
IFNβ	899-909	Forward		GTAACCTGTAAGTCTGTTAA
	453-472	Reverse	457 bp	AAGCAGCAATTTTCAGTGTC
	682-711	Probe		GAGAACCTCCTGGCTAATGTCT
	1868-1890 (exon 2)	Forward	1410 bp (genomic DNA)	CCAATTCTTCAAAATGCCTAAG
IFNγ	481-502 (exon 1)	Reverse	171 bp (mRNA)	TATACAAGTTATATCTTGGCTT
	531-558 (exon 1)	Probe		TGGCTGTTACTGCCAGGACCCATATGTA

TABLE 4

TABLE 5

GENE	Nucleotide Position	Primer	Product Size	Sequence 5' ——→ 3'
ALDOLASE A	2759-2777	Forward	181 bp (m-RNA)	CGCAGAAGGGGTCCTGGTGA
	4205-4229	Reverse		CTGGCACAGGAGAGGGGCGGGGTG
	2798-2827	Probe		TTGAATCCACTCGCCAGCCCGCCGCCCTCT

Interferon	INTERFERON TITER (U/ML)						
	Untreated	Polyclonal Anti IFN α	Monoclonal Anti IFN α2 I/B	Monoclonal Anti IFN α2 I/8	Monoclonal Anti IFN α2 I/20	Monoclonal Anti IFN ω	Monoclonal Anti IFN β
IFN α1	1000	< 3	1000	1000	1000	1000	100
IFN α2	1000	< 2	< 2	ND	ND	ND	ND
IFN α2A	24	< 2	ND	3	3	24	24
IFN α8	250	< 2	8	250	250	ND	ND
IFN ω	100	100	100	100	100	< 2	100
IFN β	100	< 2	100	100	100	100	< 2
7628	8	< 2	4	8	12	8	8
9444	16	< 2	4	16	16	16	16
10024	25	< 2	9	12	25	25	25

TABLE 6

TABLE 7

Serum	INTERFERON TITER (U/ML)							
	Untreated	Polyclonal Anti IFN α	Monoclonal Anti IFN α2	Monoclonal Anti IFN ω	Monoclonal Anti IFN β	Monoclonal Anti IFN γ	Monoclonal Anti BTP	Monoclonal Anti TNF
1274	80	< 2	40	80	160	80	80	80
1498	80	< 2	20	80	80	80	80	40
2462	80	< 2	40	80	80	80	80	80
3131	40	< 2	20	40	40	40	40	40
4506	40	< 2	10	40	40	40	40	40

TABLE 8

EFFECT OF ANTI-IFN α RECEPTOR mAb 64G12 ON THE ANTIVIRAL ACTIVITY OF HUMAN INTERFERONS

	TITRE (IFN U/ml)*	
SERUM INTERFERON FROM AIDS PATIENTS	UNTREATED	TREATED WITH mAb ANTI-IFN α R
1	75	< 2
2	50	< 2
3	150	< 2
4	75	< 2
Leukocyte IFN	50	< 2
IFN α2	50	< 2

* Human amniotic cells (WISH) were incubated in medium alone or medium containing 50 µg/ml of mAb 64G12 for 30 minutes prior to the addition of dilutions of the interferons indicated in the Table. The antiviral activity of each sample was determined alone and in the presence of the 64G12 antibody as described previously (Tovey et al., 1977, Nature, 267:455).

SUBSTITUTE SHEET (RULE 26)

	MACAQUES INFECTED WITH SIV MAC											
	471	473	475	477	483	485	493	495	499	501	505	1238
DEC 89	< 6	6	< 6	< 12	< 6	< 6	< 6	6	< 6	< 4	< 6	< 4
JAN 90						< 6				25		
FEB 90	< 6		35			< 6			< 2			
MAR 90	< 6	< 6	12			12		< 6	< 3	35		< 4
APR 90	< 6		12			< 12						
MAY 90	< 6	< 12	18		< 6	< 12		< 12	< 6*	25		< 6
JUN 90						200						
JUL 90	< 6	35	35		6	400				75		< 4
AUG 90	< 6		35		18	400	12	24	< 6			
SEP 90	< 6	18	50		50	12	12	18	< 12	75		< 6
OCT 90												
NOV 90	< 12		25		300		35				< 6	

* Two samples tested.

TABLE 9A

	MACAQUES INFECTED WITH SIV MAC											
	471	473	475	477	483	485	493	495	499	501	505	1238
DEC 90	< 6	< 6	18	< 12	400		12	< 6	< 1	35		< 6
JAN 91	< 12		12		400		18					
FEB 91							75					
MAR 91	< 1	< 12	25	< 12	200		150	< 12	< 6	50	< 6	< 25
APR 91					25							
MAY 91		6	12	< 1	50		400	4	< 1	35	< 1	< 1
JUL 91							200					
OCT 91	< 12	6	25	< 6			400	< 6	< 12	50	< 1	< 6
NOV 91	< 6	18	50					< 6		25	< 1	< 12
DEC 91	< 6			< 12					< 12			
JAN 92	< 3	25	> 200	< 3				4	< 3	200	< 3	< 3
MAR 92	< 3	12	> 200	< 3				4	< 3	50	< 3	< 3
MAY 92			100								< 3	

TABLE 9B

C 339	*	C 433	*	C 487	*	C 87	*	C 435	*
15-08-89	< 4	10-02-87	< 4	10-02-87	< 4	23-01-90	< 4	14-08-90	< 4
26-09-89	< 4	11-01-88	< 4	03-05-88	< 4	15-05-90	< 4	13-11-90	< 4
05-12-89	< 4	25-10-88	< 4	21-06-88	< 4	13-11-90	< 4	09-04-91	(2) ?
27-02-90	< 8	16-05-89	< 4	30-08-88	< 4	12-03-91	< 4	24-09-91	< 4
14-08-90	< 4	15-08-89	< 4	01-11-88	< 4	13-11-91	< 4		
25-09-90	< 4	30-01-90	< 4	01-05-89	< 4	28-01-92	< 4		
10-12-90	< 4	10-04-90	< 4	14-11-89	< 4				
13-08-91	< 4	06-11-90	< 4	08-05-90	< 4				
24-09-91	< 4	28-05-91	< 4	13-08-91	< 4				
04-02-92	(1) ?	20-08-91	< 4	04-02-92	(1) ?				
		15-10-91	< 4						
		10-12-91	< 4						

* Titre IFN (unités/ml)

TABLE 10

HLA-DR TYPING OF MIXED LYMPHOCYTE CULTURES

MLC	STIMULATOR CELLS	RESPONDER CELLS
1	DR 2 - 4	DR 3 - 6
2	DR 3 - 6	DR 2 - 4
3	DR 1 - 7	DR 4 - 5
4	DR 4 - 5	DR 1 - 7
5	DR 2 - 3	DR 7
6	DR 7	DR 2 - 3
7	DR 2 - 3	DR 1 - 5
8	DR 1 - 5	DR 2 - 3
9	DR 6	DR 1 - 5
10	DR 1 - 5	DR 6
11	DR 1 - 7	DR 6
12	DR 6	DR 1 - 7

Peripheral blood mononuclear cells from normal donors were typed for MHC class I and class II antigens by complement dependent microlymphocytotoxicity for each pair of cultures.

TABLE 11

TABLE 12

SKIN ALLOGRAFT IN SURVIVAL CYNOMOLOGUS MONKEYS

	M.S.T. (Days)
Untreated Control group	7.5 ± 0.57 (n = 4)
Cyclosporin A (5.0 mg/Kg/day)	9.5 ± 0.57 (n = 4)
Anti-IFN α receptor mAb IgG	14.25 ± 0.95 (n = 4)
Cyclosporin A + anti-IFN receptor mAb IgG	63 (n = 1) > 325 n = 4 *

* Treatment with the antibody 64G12 and cyclosporin A was stopped at days 85 and 140 respectively

TABLE 13

EXPRESSION OF MHC CLASS I AND CLASS II ANTIGENS ON THE GRAFTED TISSUE

TREATMENT	MHC CLASS I ANTIGENS					MHC CLASS II ANTIGENS				
	Day 5	10	20	60	180	Day 5	10	20	60	180
UNTREATED	+++	R	R	R	R	+++	R	R	R	R
CyA	+++	+++	R	R	R	++	++	R	R	R
64G12	++	++	R	R	R	+++	+++	R	R	R
CyA+G64G12	+	+	+	+	+	++	+	+	+	+

The degree of expression of MHC antigens was defined as : + weak ; ++ moderate ; +++ strong ;
R = reject

% LYMPHOCYTES

	TIME (DAYS)				
	0	5	15	30	60
Untrealed Control group	27±1.4	29.2±4.6	24.2±3.8	26.7±3.8	34.5±7.7
Cyclosporin A (5.0 mg/Kg/day)	35±12.7	42.5±16	35.7±10.4	28.5±10.8	24.5±7.7
Anti-IFN α R mAb IgG	39.5±12	59.2±9.7	64.5±4.4	56.5±8.5	66.5±3.5
Cyclosporin A + anti-IFN R mAb IgG	26.5±2.1	61.8±7.9	62.3±5	61.4±2	63±2.1

TABLE 14

SKIN ALLOGRAFT SURVIVAL IN CYNOMOLOGUS MONKEYS

TREATMENT	% CD_4^+ CELLS	% CD_8^+ CELLS
Untreated	23.3 ± 7.5	33.65 ± 2.75
Treated with anti-IFN α mAb receptor	25.5 ± 2.7	51.45 ± 5.0

The number of CD_4^+ T lymphocytes and CD_8^+T lymphocytes was determined by FACS-SCAN using phycoerythrin conjugated anti-human CD_4^+ monoclonal antibody (OTK4 Ortho Diagnostics), and anti-human Leu2a monoclonal antibody (Becton-Dickinson) respectively.

TABLE 15

TABLE 16A

GROUP	PRIMATE	CREATININ mmol/l					SODIUM mmol/l					POTASSIUM mmol/l				
		J0	J5	J15	J30	J60	J0	J5	J15	J30	J60	J0	J5	J15	J30	J60
I	1		80	87	83	72		146	144	138	132		4	4,3	3,9	3,7
	2		115	90	87	90		146	152	142	157		4,4	5,1	5	3,9
	3	98	91	86	90		142	146	146	153		3,5	3,9	3,7	4	
	4	120	89	89	101		145	142	143	129		4,1	3,9	3,8	4,2	
MEAN		109	93,75	88	90,25	81	143,5	145	146,25	140,5	144,5	3,8	4,05	4,23	4,28	3,8
STANDARD DEVIATION		15,56	14,95	1,83	7,72	12,73	2,12	2	4,03	9,95	17,68	0,42	0,24	0,64	0,50	0,14
II	1		104	82	96	89		142	142	142	144		3,9	4,1	3,7	3,9
	2		122	89	92	88		154	146	141	139		4,9	5	4,7	4,1
	3	118	106	88	93		143	145	142	142		4,1	4,3	4,2	4,4	
	4	94	107	94	90		140	147	150	145		5,1	4,9	4,1	4,4	
MEAN		106	109,75	88,25	92,75	88,5	141,5	147	145	142,5	141,5	4,6	4,5	4,35	4,3	4
STANDARD DEVIATION		16,97	8,26	4,92	2,5	0,71	2,12	5,10	3,83	1,73	3,54	0,71	0,49	0,44	0,42	0,14

Table: EVOLUTION OF THE LEVELS OF PLASMATIC CREATININ, NA^+ AND K^+ IN THE 4 GROUPS STUDIED AT J0, J5, J15, J30 AND J60 (CORRESPONDING TO DAY 0, DAY 5, DAY 15, DAY 30 AND DAY 60).

TABLE 16B

GROUP	PRIMATE	CREATININ mmol/l					SODIUM mmol/l					POTASSIUM mmol/l				
		J0	J5	J15	J30	J60	J0	J5	J15	J30	J60	J0	J5	J15	J30	J60
III	1		83	68	125	84		145	144	142	140		4,1	3,9	4,2	4,4
	2		91	103	80	120		149	143	143	143		3,7	4,1	3,9	4,2
	3	102	88	95	84		139	150	147	140		4,6	5,3	4,7	4,1	
	4	72	67	82	102		141	145	151	139		4,2	4,5	4,1	3,9	
MEAN		87	82,25	87	97,75	102	140	147,25	146,25	141	141,5	4,4	4,4	4,2	4,03	4,3
STANDARD DEVIATION		21,21	10,69	15,34	20,53	25,46	1,41	2,62996	3,59	1,83	2,12	0,28	0,68	0,35	0,15	0,14
IV	1		90	93	89	88		139	140	141	137		4,2	4,3	3,9	4,1
	2		85	69				129	144				4,7	4,8		
	3		91	107	87	62		145	147	139	135		4,3	4,3	4,5	4,4
	4		69	123	126	105		151	149	144	147		4,9	4,4	4,7	3,9
	5	93	69	90	101		131	144	145	144		5,1	5,2	4,9	4,4	
	6	99	92	103	85		139	144	142	132		4,3	4,5	4,4	4,6	
MEAN		96	82,67	97,5	97,6	85	135	142	144,5	140	139,667	4,7	4,63	4,52	4,42	4,13
STANDARD DEVIATION		4,24	10,86	18,22	17,05	21,66	5,66	7,43	3,27	4,95	6,43	0,57	0,38	0,26	0,31	0,25

Table: EVOLUTION OF THE LEVELS OF PLASMATIC CREATININ, NA^+ AND K^+ IN THE 4 GROUPS STUDIED AT J0, J5, J15, J30 AND J60 (CORRESPONDING TO DAY 0, DAY 5, DAY 15, DAY 30 AND DAY 60).

SERUM CYCLOSPORIN LEVELS (ng/ml)

	TIME (DAYS)			
	5	15	30	60
Cyclosporin A (5mg/Kg/day)	123±27	145±89	181±83	180±42
Cyclosporin A + anti-IFN receptor mAb IgG	301±132	321±83	377±78	285±71

TABLE 17

TABLE 18

SURVIVAL OF SECONDARY SKIN GRAFTS IN CYNOMOLOGUS MONKEYS

	M.S.T. (Days)
Untreated Control group	5.5 ± 0.50 (n = 4)
Cyclosporin A + anti-IFN receptor mAb IgG	11.5 ± 1.5 (n = 4)*

* Animals were grafted with skin from the same donor 226 days after primary grafting without any further treatment. (Animals had been treated previously with antibody 64G12 and cyclosporin A for 85 and 140 days respectively).

TABLE 19

GRAFT VERSUS HOST DISEASE

	M.S.T. (Days)
Untreated control group	9 ± 1.0 (n = 4)
Cyclosporin A + anti-IFN receptor mAb IgG	19 * (n = 1)
	> 93 (n = 2)

* Died from an infection without clinical or histological signs of GVH

CLAIMS

1. Pharmaceutical composition, for use as immunomodulator, characterized in that it comprises a purified preparation of monoclonal antibodies directed against the human interferon class I receptor (IFN-R) and having the following properties :
   - it recognizes the extracellular domain of the human IFN-R, and
   - it has a neutralizing capacity against the biological properties of the human type I-IFN.

2. Pharmaceutical composition according to claim 1 wherein the monoclonal antibody is characterized by its capacity to inhibit the binding of a human pathological type I-IFN, to the type I IFN-R.

3. Pharmaceutical composition according to claim 1 or 2, wherein the monoclonal antibody is obtainable from a hybridoma cell prepared by fusion of a myeloma cell with spleen cells from an animal previously immunized with the soluble form of the human IFN-R.

4. Pharmaceutical composition according to anyone of claims 1, 2 or 3, wherein the monoclonal antibody recognizes an epitope on a soluble form of the human cellular IFN-R or of a recombinant IFN-R.

5. Pharmaceutical composition according to anyone of claims 1 to 4, wherein the monoclonal antibody inhibits in vitro the binding of human type I-IFN, to the human cellular IFN-R when it is co-incubated with cells harboring the hu-IFN-R, at a concentration of antibodies equal or inferior to 100 µg/ml, preferably equal or inferior to 50 µg/ml, advantageously inferior to 20 µg/ml, more preferably in the range of approximately 0,5 to 2 µg/ml.

6. Pharmaceutical composition according to anyone of claims 1 to 5, wherein the monoclonal antibody

neutralizes in vitro the antiproliferative activity of the human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 10 μg/ml.

7. Pharmaceutical composition according to anyone of claims 1 to 6, wherein the monoclonal antibody neutralizes in vitro the antiproliferative activity of human type I-IFN, on cells poorly responsive to this human type I-IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 μg/ml.

8. Pharmaceutical composition according to anyone of claims 1 to 7, wherein the monoclonal antibody does not bind to the human receptor of the IFN gamma.

9. Pharmaceutical composition according to anyone of claims 1 to 8, wherein the monoclonal antibody recognizes an epitope on the aminoacid sequence 27 to 427 of the human IFN-R.

10. Pharmaceutical composition according to anyone of claims 1 to 9, wherein the monoclonal antibody neutralizes in vitro the antiviral activity of the human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 10 μg/ml.

11. Pharmaceutical composition according to anyone of claims 1 to 10, wherein the monoclonal antibody neutralizes in vitro the antiviral activity of the human class I-IFN, on cells poorly responsive to this human IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 μg/ml.

12. Pharmaceutical composition according to anyone of claims 1 to 11, for use as immunosuppressor.

13. Pharmaceutical composition according to anyone of claims 1 to 11, for use as immunostimulator.

14. Pharmaceutical composition according to anyone of claims 1 to 13, wherein the monoclonal antibody is the 64G12 antibody, deposited at the ECACC on February 26, 1992 under n° 92022605.

15. Pharmaceutical composition according to anyone of claims 1 to 13, wherein the monoclonal antibody is a humanized antibody, for instance characterized in that the variable or complementary determining regions of its heavy and light chains are grafted on the framework and constant regions of a human antibody.

16. Pharmaceutical composition according to anyone of claims 1 to 13, wherein the monoclonal antibody is a human antibody.

17. Pharmaceutical composition according to anyone of claims 1 to 16, characterized in that the purified preparation of monoclonal antibodies is used at a dose between around 0.05 mg/kg of bodyweight and around 3 mg/kg.

18. Pharmaceutical composition according to anyone of claims 1 to 17 comprising a pharmaceutical vehicle appropriate for the intravenous administration.

19. Pharmaceutical composition according to anyone of claims 1 to 17 comprising a pharmaceutical vehicle appropriate for the intramuscular administration.

20. Use of a purified preparation of monoclonal antibodies as defined in anyone of claims 1 to 16, for the preparation of a drug having an immunomodulator effect sufficient to inhibit in vivo the disease due to the infection by a human retrovirus especially by a human HIV or HTLV retrovirus.

21. Use of a purified preparation of monoclonal antibodies as defined in anyone of claims 1 to 16, for the preparation of a drug having an immunomodulator

effect sufficient to inhibit in vivo the rejection of allografts.

22. Use of a purified preparation of monoclonal antibodies as defined in anyone of claims 1 to 16, for the preparation of a drug having an immunomodulator effect sufficient to inhibit in vivo the symptoms of the Graft Versus Host Disease.

23. Pharmaceutical composition for use as immunosuppressor, characterized in that it comprises as active principle, monoclonal antibodies as defined in anyone of claims 1 to 16, and as a combined preparation for simultaneous, separate or sequential use, an agent having an immunomodulator activity especially an immunosuppressor activity.

24. Pharmaceutical composition according to claim 23, characterized in that the agent having an immunomodulator activity is cyclosporin A or FK 506.

25. Pharmaceutical composition according to claim 19, characterized in that the monoclonal antibodies are present at a dose ranging from around 0.05 mg/kg to 3 mg/kg, preferably from 0.5 mg/kg to 1 mg/kg and the agent having immunosuppressor activity, especially cyclosporin A, is present in a dose of at least 0.1 mg/kg.

26. Use of a purified preparation of monoclonal antibodies as defined in anyone of claims 1 to 16, as adjuvant for humoral or cellular immunity.

27. Use of a purified preparation of monoclonal antibodies as defined in anyone of claims 1 to 16, for the preparation of a drug applicable to the treatment of autoimmune disease, particularly those diseases characterized by the abnormal or prolonged production of type I-IFN, especially α IFN.

28. Use of a preparation of monoclonal antibodies as defined in anyone of claims 1 to 16, for the in vitro detection, on a biological sample such as blood or another biological fluid, of the presence of soluble type I interferon receptors, especially in case of autoimmune diseases or HIV infection.

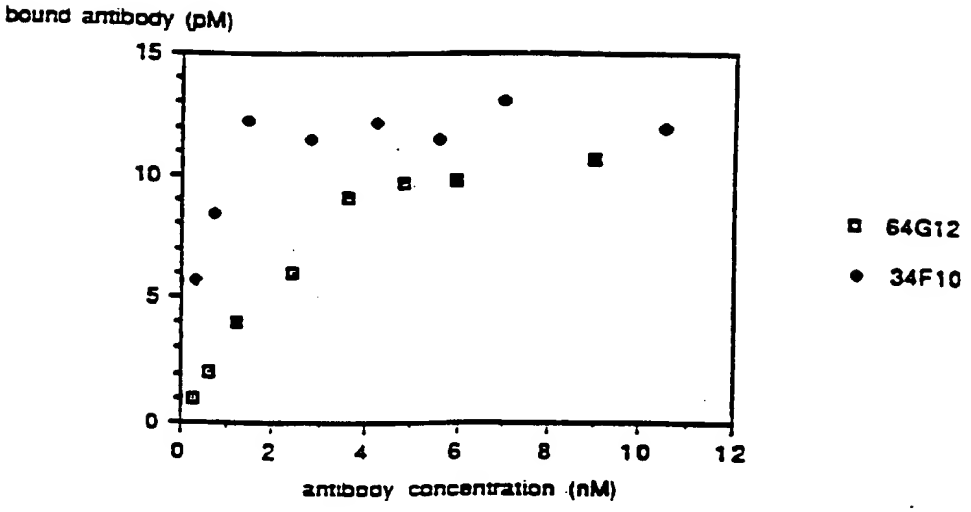


FIGURE 1A

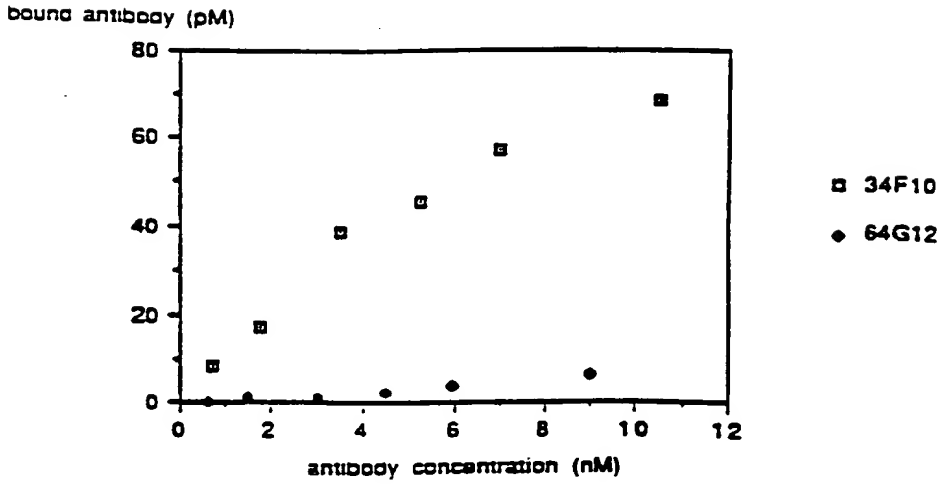


FIGURE 1B

CTGCAGGGATCTGCGGCGGCTCCCAG

ATG ATG GTC GTC CTC CTG GGC GCG ACG ACC CTA GTG CTC GTC GCC GTG GGC CCA
Met Met Val Val Leu Leu Gly Ala Thr Thr Leu Val Leu Val Ala Val Gly Pro

TGG GTG TTG TCC GCA GCC GCA GGT GGA AAA AAT CTA AAA TCT CCT CAA AAA GTA
Trp Val Leu Ser Ala Ala Ala Gly Gly Lys Asn Leu Lys Ser Pro Gln Lys Val

GAG GTC GAC ATC ATA GAT GAC AAC TTT ATC CTG AGG TGG AAC AGG AGC GAT GAG
Glu Val Asp Ile Ile Asp Asp Asn Phe Ile Leu Arg Trp Asn Arg Ser Asp Glu

TCT GTC GGG AAT GTG ACT TTT TCA TTC GAT TAT CAA AAA ACT GGG ATG GAT AAT
Ser Val Gly Asn Val Thr Phe Ser Phe Asp Tyr Gln Lys Thr Gly Met Asp Asn

TGG ATA AAA TTG TCT GGG TGT CAG AAT ATT ACT AGT ACC AAA TGC AAC TTT TCT
Trp Ile Lys Leu Ser Gly Cys Gln Asn Ile Thr Ser Thr Lys Cys Asn Phe Ser

TCA CTC AAG CTG AAT GTT TAT GAA GAA ATT AAA TTG CGT ATA AGA GCA GAA AAA
Ser Leu Lys Leu Asn Val Tyr Glu Glu Ile Lys Leu Arg Ile Arg Ala Glu Lys

GAA AAC ACT TCT TCA TGG TAT GAG GTT GAC TCA TTT ACA CCA TTT CGC AAA GCT
Glu Asn Thr Ser Ser Trp Tyr Glu Val Asp Ser Phe Thr Pro Phe Arg Lys Ala

CAG ATT GGT CCT CCA GAA GTA CAT TTA GAA GCT GAA GAT AAG GCA ATA GTG ATA
Gln Ile Gly Pro Pro Glu Val His Leu Glu Ala Glu Asp Lys Ala Ile Val Ile

CAC ATC TCT CCT GGA ACA AAA GAT AGT GTT ATG TGG GCT TTG GAT GGT TTA AGC
His Ile Ser Pro Gly Thr Lys Asp Ser Val Met Trp Ala Leu Asp Gly Leu Ser

TTT ACA TAT AGC TTA CTT ATC TGG AAA AAC TCT TCA GGT GTA GAA GAA AGG ATT
Phe Thr Tyr Ser Leu Leu Ile Trp Lys Asn Ser Ser Gly Val Glu Glu Arg Ile

GAA AAT ATT TAT TCC AGA CAT AAA ATT TAT AAA CTC TCA CCA GAG ACT ACT TAT
Glu Asn Ile Tyr Ser Arg His Lys Ile Tyr Lys Leu Ser Pro Glu Thr Thr Tyr

TGT CTA AAA GTT AAA GCA GCA CTA CTT ACG TCA TGG AAA ATT GGT GTC TAT AGT
Cys Leu Lys Val Lys Ala Ala Leu Leu Thr Ser Trp Lys Ile Gly Val Tyr Ser

CCA GTA CAT TGT ATA AAG ACC ACA GTT GAA AAT GAA CTA CCT CCA CCA GAA AAT
Pro Val His Cys Ile Lys Thr Thr Val Glu Asn Glu Leu Pro Pro Pro Glu Asn

ATA GAA GTC AGT GTC CAA AAT CAG AAC TAT GTT CTT AAA TGG GAT TAT ACA TAT
Ile Glu Val Ser Val Gln Asn Gln Asn Tyr Val Leu Lys Trp Asp Tyr Thr Tyr

GCA AAC ATG ACC TTT CAA GTT CAG TGG CTC CAC GCC TTT TTA AAA AGG AAT CCT
Ala Asn Met Thr Phe Gln Val Gln Trp Leu His Ala Phe Leu Lys Arg Asn Pro

GGA AAC CAT TTG TAT AAA TGG AAA CAA ATA CCT GAC TGT GAA AAT GTC AAA ACT
Gly Asn His Leu Tyr Lys Trp Lys Gln Ile Pro Asp Cys Glu Asn Val Lys Thr

ACC CAG TGT GTC TTT CCT CAA AAC GTT TTC CAA AAA GGA ATT TAC CTT CTC CGC
Thr Gln Cys Val Phe Pro Gln Asn Val Phe Gln Lys Gly Ile Tyr Leu Leu Arg

GTA CAA GCA TCT GAT GGA AAT AAC ACA TCT TTT TGG TCT GAA GAG ATA AAG TTT
Val Gln Ala Ser Asp Gly Asn Asn Thr Ser Phe Trp Ser Glu Glu Ile Lys Phe

GAT ACT GAA ATA CAA GCT TTC CTA CTT CCT CCA GTC TTT AAC ATT AGA TCC CTT
Asp Thr Glu Ile Gln Ala Phe Leu Leu Pro Pro Val Phe Asn Ile Arg Ser Leu

FIGURE 2A

```
AGT GAT TCA TTC CAT ATC TAT ATC GGT GCT CCA AAA CAG TCT GGA AAC ACG CCT
Ser Asp Ser Phe His Ile Tyr Ile Gly Ala Pro Lys Gln Ser Gly Asn Thr Pro

GTG ATC CAG GAT TAT CCA CTG ATT TAT GAA ATT ATT TTT TGG GAA AAC ACT TCA
Val Ile Gln Asp Tyr Pro Leu Ile Tyr Glu Ile Ile Phe Trp Glu Asn Thr Ser

AAT GCT GAG AGA AAA ATT ATC GAG AAA AAA ACT GAT GTT ACA GTT CCT AAT TTG
Asn Ala Glu Arg Lys Ile Ile Glu Lys Lys Thr Asp Val Thr Val Pro Asn Leu

AAA CCA CTG ACT GTA TAT TGT GTG AAA GCC AGA GCA CAC ACC ATG GAT GAA AAG
Lys Pro Leu Thr Val Tyr Cys Val Lys Ala Arg Ala His Thr Met Asp Glu Lys

CTG AAT AAA AGC AGT GTT TTT AGT GAC GCT GTA TGT GAG AAA ACA AAA CCA GGA
Leu Asn Lys Ser Ser Val Phe Ser Asp Ala Val Cys Glu Lys Thr Lys Pro Gly

AAT ACC TCT AAA TGA GGT ACC
Asn Thr Ser Lys
```

FIGURE 2B

CTGCAGGGATCTGCGGCGGCTCCCAG

ATG ATG GTC GTC CTC CTG GGC GCG ACG ACC CTA GTG CTC GTC GCC GTG GGC CCA
Met Met Val Val Leu Leu Gly Ala Thr Thr Leu Val Leu Val Ala Val Gly Pro

TGG GTG TTG TCC GCA GCC GCA GGT GGA AAA AAT CTA AAA TCT CCT CAA AAA GTA
Trp Val Leu Ser Ala Ala Ala Gly Gly Lys Asn Leu Lys Ser Pro Gln Lys Val

GAG GTC GAC ATC ATA GAT GAC AAC TTT ATC CTG AGG TGG AAC AGG AGC GAT GAG
Glu Val Asp Ile Ile Asp Asp Asn Phe Ile Leu Arg Trp Asn Arg Ser Asp Glu

TCT GTC GGG AAT GTG ACT TTT TCA TTC GAT TAT CAA AAA ACT GGG ATG GAT AAT
Ser Val Gly Asn Val Thr Phe Ser Phe Asp Tyr Gln Lys Thr Gly Met Asp Asn

TGG ATA AAA TTG TCT GGG TGT CAG AAT ATT ACT AGT ACC AAA TGC AAC TTT TCT
Trp Ile Lys Leu Ser Gly Cys Gln Asn Ile Thr Ser Thr Lys Cys Asn Phe Ser

TCA CTC AAG CTG AAT GTT TAT GAA GAA ATT AAA TTG CGT ATA AGA GCA GAA AAA
Ser Leu Lys Leu Asn Val Tyr Glu Glu Ile Lys Leu Arg Ile Arg Ala Glu Lys

GAA AAC ACT TCT TCA TGG TAT GAG GTT GAC TCA TTT ACA CCA TTT CGC AAA GCT
Glu Asn Thr Ser Ser Trp Tyr Glu Val Asp Ser Phe Thr Pro Phe Arg Lys Ala

CAG ATT GGT CCT CCA GAA GTA CAT TTA GAA GCT GAA GAT AAG GCA ATA GTG ATA
Gln Ile Gly Pro Pro Glu Val His Leu Glu Ala Glu Asp Lys Ala Ile Val Ile

CAC ATC TCT CCT GGA ACA AAA GAT AGT GTT ATG TGG GCT TTG GAT GGT TTA AGC
His Ile Ser Pro Gly Thr Lys Asp Ser Val Met Trp Ala Leu Asp Gly Leu Ser

TTT ACA TAT AGC TTA CTT ATC TGG AAA AAC TCT TCA GGT GTA GAA GAA AGG ATT
Phe Thr Tyr Ser Leu Leu Ile Trp Lys Asn Ser Ser Gly Val Glu Glu Arg Ile

GAA AAT ATT TAT TCC AGA CAT AAA ATT TAT AAA CTC TCA CCA GAG ACT ACT TAT
Glu Asn Ile Tyr Ser Arg His Lys Ile Tyr Lys Leu Ser Pro Glu Thr Thr Tyr

TGT CTA AAA GTT AAA GCA GCA CTA CTT ACG TCA TGG AAA ATT GGT GTC TAT AGT
Cys Leu Lys Val Lys Ala Ala Leu Leu Thr Ser Trp Lys Ile Gly Val Tyr Ser

CCA GTA CAT TGT ATA AAG ACC ACA GTT GAA AAT GAA CTA CCT CCA CCA GAA AAT
Pro Val His Cys Ile Lys Thr Thr Val Glu Asn Glu Leu Pro Pro Pro Glu Asn

ATA GAA GTC AGT GTC CAA AAT CAG AAC TAT GTT CTT AAA TGG GAT TAT ACA TAT
Ile Glu Val Ser Val Gln Asn Gln Asn Tyr Val Leu Lys Trp Asp Tyr Thr Tyr

GCA AAC ATG ACC TTT CAA GTT CAG TGG CTC CAC GCC TTT TTA AAA AGG AAT CCT
Ala Asn Met Thr Phe Gln Val Gln Trp Leu His Ala Phe Leu Lys Arg Asn Pro

GGA AAC CAT TTG TAT AAA TGG AAA CAA ATA CCT GAC TGT GAA AAT GTC AAA ACT
Gly Asn His Leu Tyr Lys Trp Lys Gln Ile Pro Asp Cys Glu Asn Val Lys Thr

ACC CAG TGT GTC TTT CCT CAA AAC GTT TTC CAA AAA GGA ATT TAC CTT CTC CGC
Thr Gln Cys Val Phe Pro Gln Asn Val Phe Gln Lys Gly Ile Tyr Leu Leu Arg

GTA CAA GCA TCT GAT GGA AAT AAC ACA TCT TTT TGG TCT GAA GAG ATA AAG TTT
Val Gln Ala Ser Asp Gly Asn Asn Thr Ser Phe Trp Ser Glu Glu Ile Lys Phe

GAT ACT GAA ATA CAA GCT TTC CTA CTT CCT CCA GTC TTT AAC ATT AGA TCC CTT
Asp Thr Glu Ile Gln Ala Phe Leu Leu Pro Pro Val Phe Asn Ile Arg Ser Leu

FIGURE 3A

```
AGT GAT TCA TTC CAT ATC TAT ATC GGT GCT CCA AAA CAG TCT GGA AAC ACG CCT
Ser Asp Ser Phe His Ile Tyr Ile Gly Ala Pro Lys Gln Ser Gly Asn Thr Pro

GTG ATC CAG GAT TAT CCA CTG ATT TAT GAA ATT ATT TTT TGG GAA AAC ACT TCA
Val Ile Gln Asp Tyr Pro Leu Ile Tyr Glu Ile Ile Phe Trp Glu Asn Thr Ser

AAT GCT GAG AGA AAA ATT ATC GAG AAA AAA ACT GAT GTT ACA GTT CCT AAT TTG
Asn Ala Glu Arg Lys Ile Ile Glu Lys Lys Thr Asp Val Thr Val Pro Asn Leu

AAA CCA CTG ACT GTA TAT TGT GTG AAA GCC AGA GCA CAC ACC ATG GAT GAA AAG
Lys Pro Leu Thr Val Tyr Cys Val Lys Ala Arg Ala His Thr Met Asp Glu Lys

CTG AAT AAA AGC AGT GTT TTT AGT GAC GCT GTA TGT GAG AAA ACA AAA CCA GGA
Leu Asn Lys Ser Ser Val Phe Ser Asp Ala Val Cys Glu Lys Thr Lys Pro Gly

AAT ACC TCT AAA ATT TGG CTT ATA GTT GGA ATT TGT ATT GCA TTA TTT GCT CTC
Asn Thr Ser Lys Ile Trp Leu Ile Val Gly Ile Cys Ile Ala Leu Phe Ala Leu

CCG TTT GTC ATT TAT GCT GCG AAA GTC TTC TTG AGA TGC ATC AAT TAT GTC TTC
Pro Phe Val Ile Tyr Ala Ala Lys Val Phe Leu Arg Cys Ile Asn Tyr Val Phe

TTT CCA TCA CTT AAA CCT TCT TCC AGT ATA GAT GAG TAT TTC TCT GAA CAG CCA
Phe Pro Ser Leu Lys Pro Ser Ser Ser Ile Asp Glu Tyr Phe Ser Glu Gln Pro

TTG AAG AAT CTT CTG CTT TCA ACT TCT GAG GAA CAA ATC GAA AAA TGT TTC ATA
Leu Lys Asn Leu Leu Leu Ser Thr Ser Glu Glu Gln Ile Glu Lys Cys Phe Ile

ATT GAA AAT ATA AGC ACA ATT GCT ACA GTA GAA GAA ACT AAT CAA ACT GAT GAA
Ile Glu Asn Ile Ser Thr Ile Ala Thr Val Glu Glu Thr Asn Gln Thr Asp Glu

GAT CAT AAA AAA TAC AGT TCC CAA ACT AGC CAA GAT TCA GGA AAT TAT TCT AAT
Asp His Lys Lys Tyr Ser Ser Gln Thr Ser Gln Asp Ser Gly Asn Tyr Ser Asn

GAA GAT GAA AGC GAA AGT AAA ACA AGT GAA GAA CTA CAG CAG GAC TTT GTA TGA
Glu Asp Glu Ser Glu Ser Lys Thr Ser Glu Glu Leu Gln Gln Asp Phe Val

CCAGAAATGAACTGTGTCAAGTATAAGGTTTTTCAGCAGGAGTTACACTGGTACC
```

FIGURE 3B

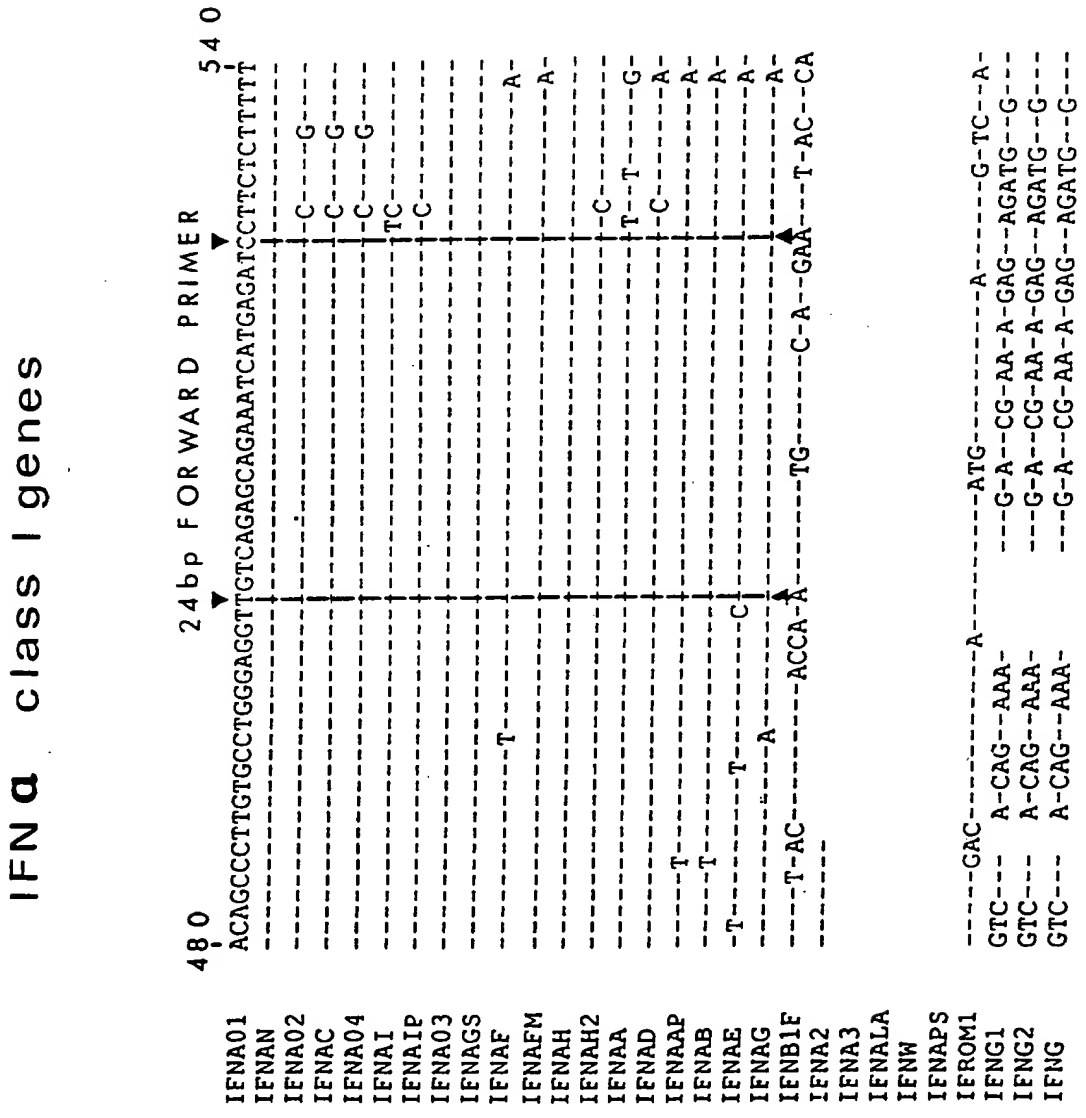
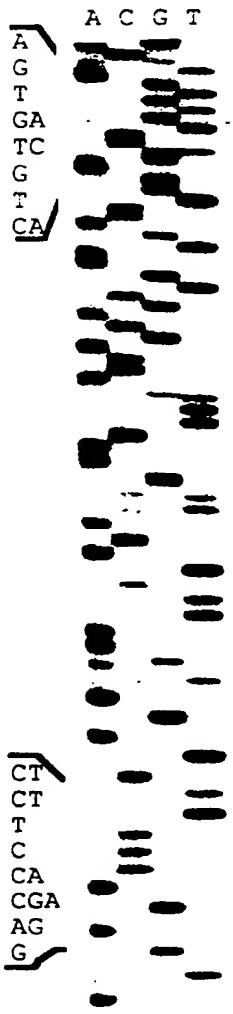

IFN α class I genes
480
24bp FORWARD PRIMER
540
IFNA01 ACAGCCCTTGTGCCTGGGAGGTTGTCAGAGCAGAAATCATGAGATCCTTCTCTTTTT
IFNAN
IFNA02
IFNAC
IFNA04
IFNAI
IFNAIP
IFNA03
IFNAGS
IFNAF
IFNAFM
IFNAH
IFNAH2
IFNAA
IFNAD
IFNAAP
IFNAB
IFNAE
IFNAG
IFNB1F
IFNA2
IFNA3
IFNALA
IFNW
IFNAPS
IFROM1
IFNG1
IFNG2
IFNG

FIGURE 4

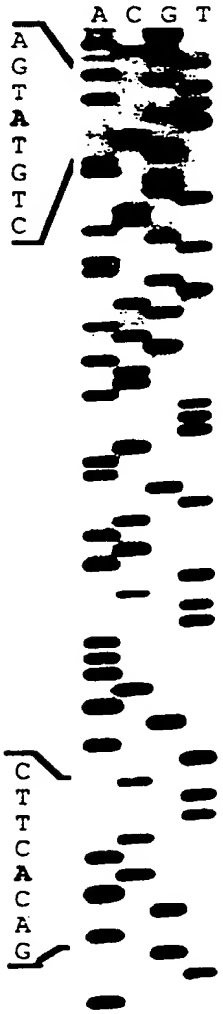
SELECTIVE PRODUCTION OF INTERFERON α SUBTYPES IN AIDS PATIENTS

Interferon alpha n



Sample of peripheral leukocytes blood from a renal allograft patient with a CMV infection

Interferon alpha 5



Sample of peripheral blood leukocytes from an AIDS patient without detectable opportunistic infections

FIGURE 5

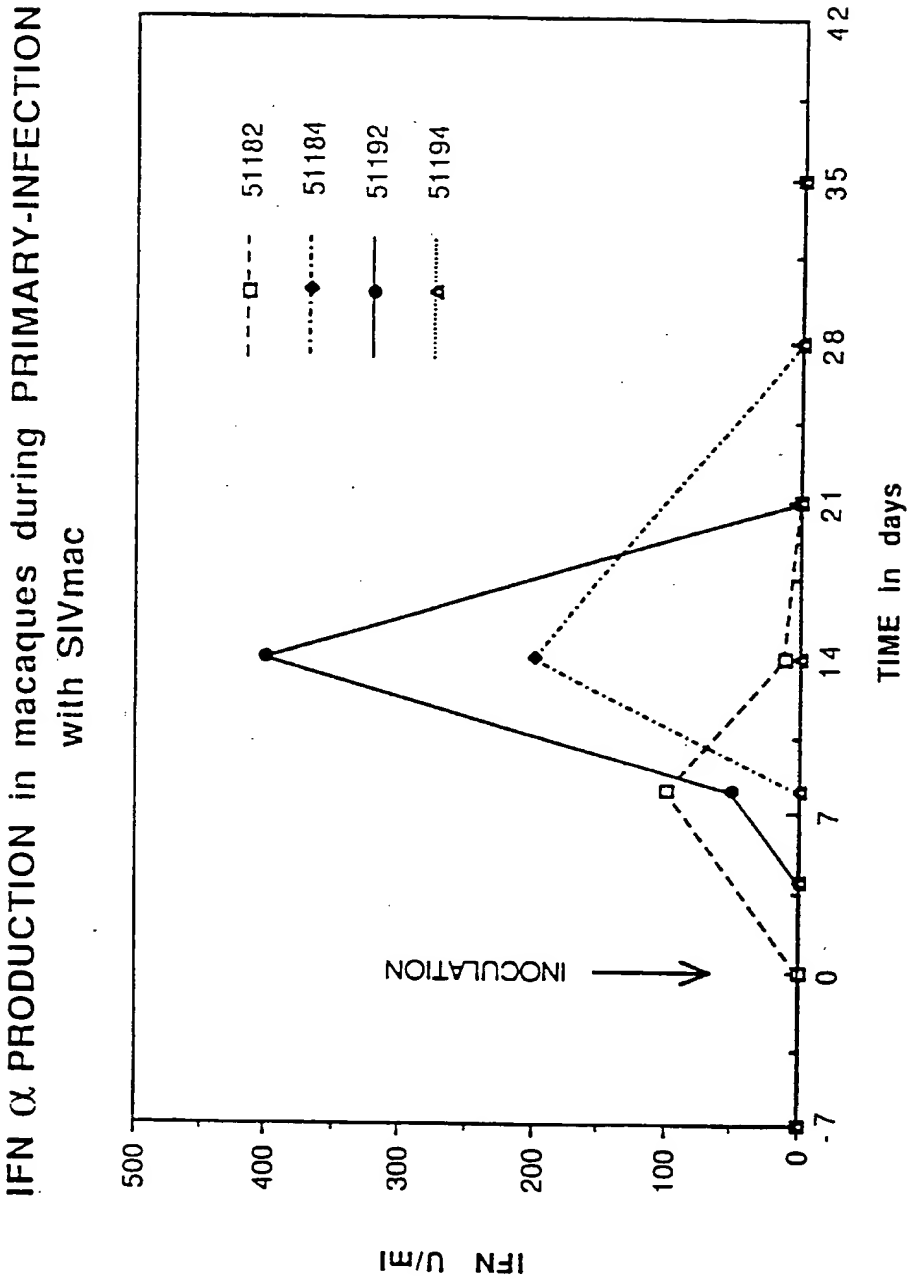

IFN α PRODUCTION in macaques during PRIMARY-INFECTION with SIVmac
IFN U/ml
500
400
300
200
100
0
-7
0
7
14
21
28
35
42
TIME in days
INOCULATION
51182
51184
51192
51194

FIGURE 6

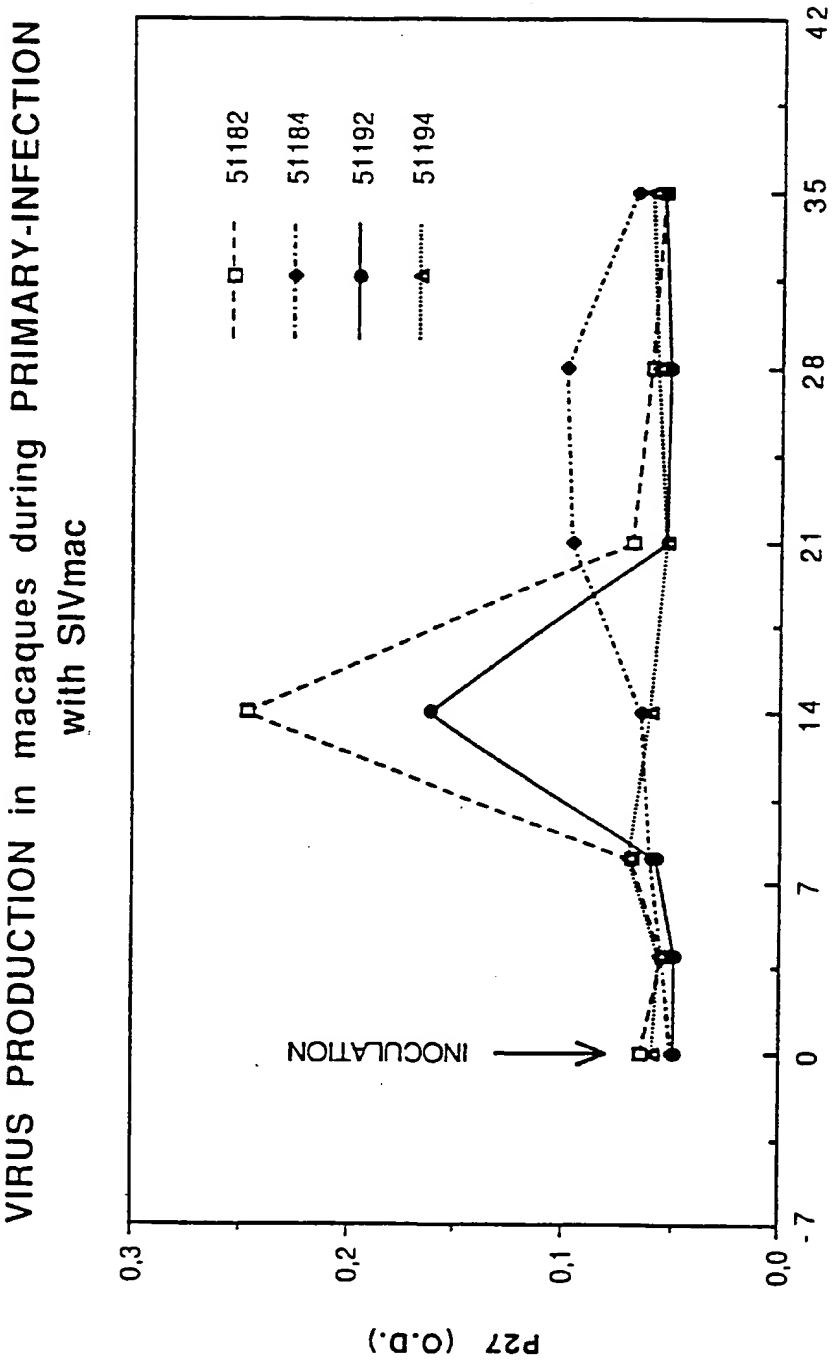

VIRUS PRODUCTION in macaques during PRIMARY-INFECTION
with SIVmac
51182
51184
51192
51194
INOCULATION
P27 (O.D.)
0,0
0,1
0,2
0,3
-7
0
7
14
21
28
35
42
TIME in days

FIGURE 7

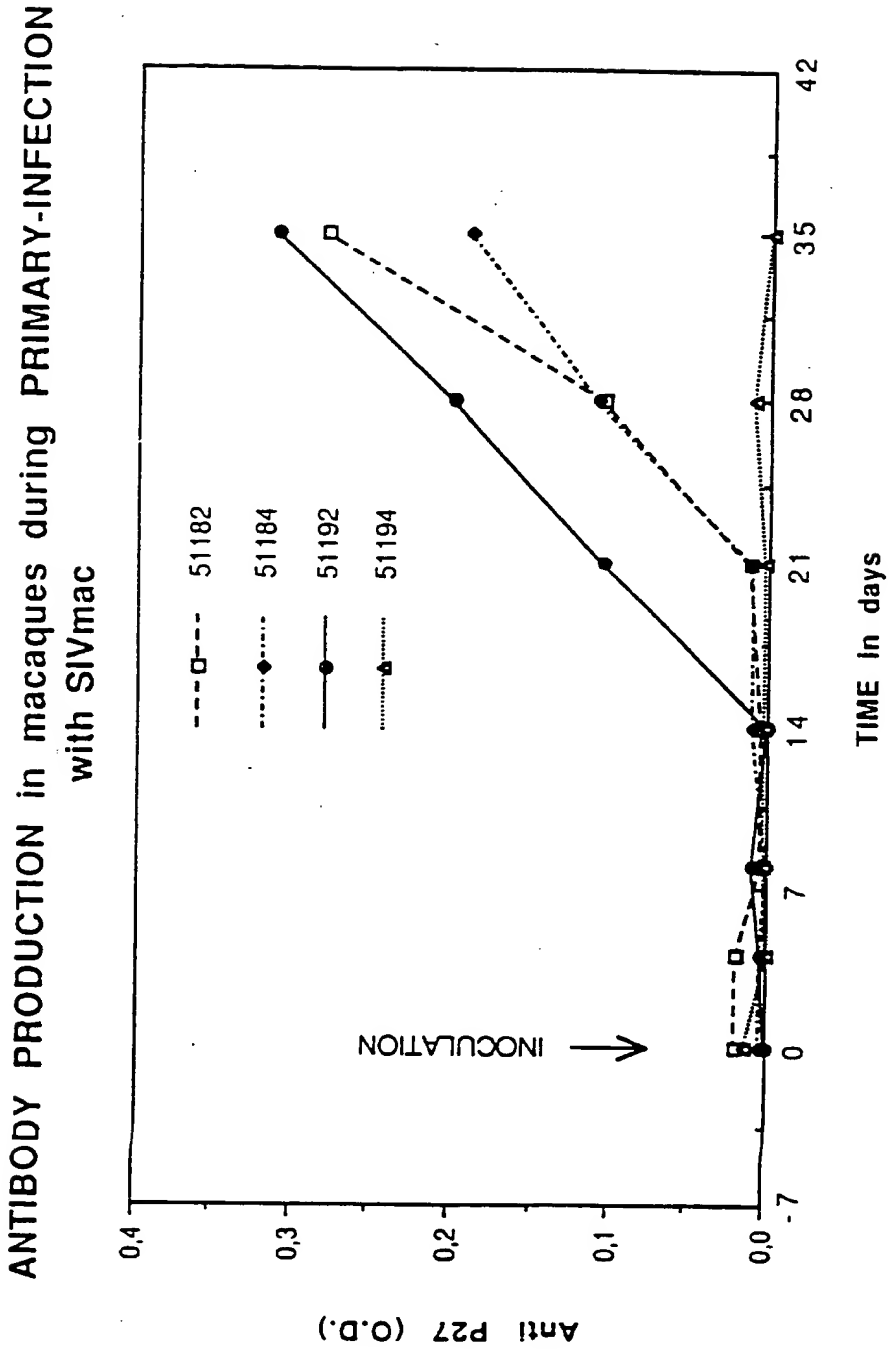

ANTIBODY PRODUCTION in macaques during PRIMARY-INFECTION with SIVmac
Anti P27 (O.D.)
0,0
0,1
0,2
0,3
0,4
-7
0
7
14
21
28
35
42
TIME in days
INOCULATION
51182
51184
51192
51194

FIGURE 8

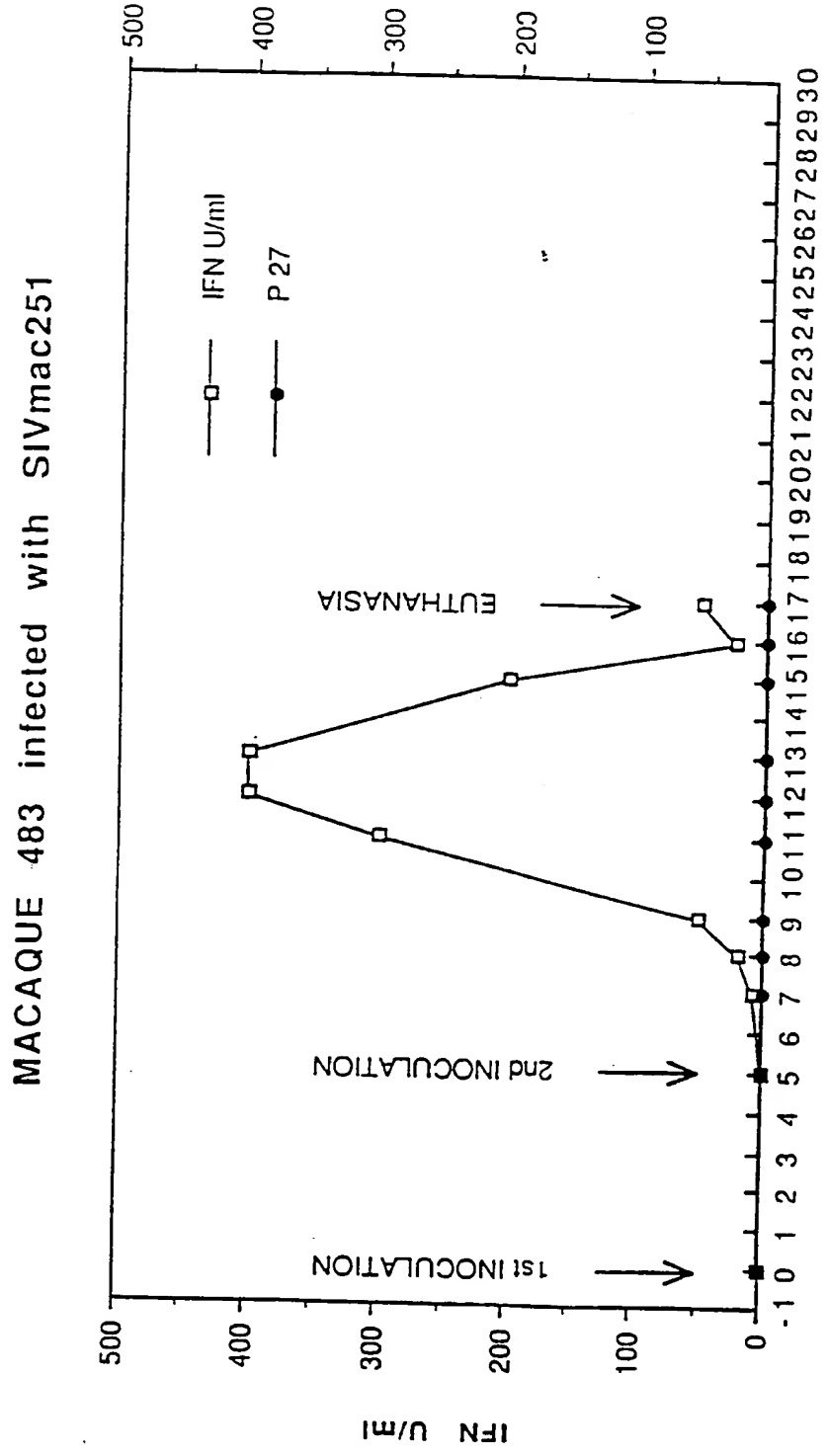

MACAQUE 483 infected with SIVmac251
IFN U/ml
P 27 (pg/ml)
IFN U/ml
P 27
1st INOCULATION
2nd INOCULATION
EUTHANASIA
MONTHS POST INFECTION
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
0 100 200 300 400 500
100 200 300 400 500

FIGURE 9

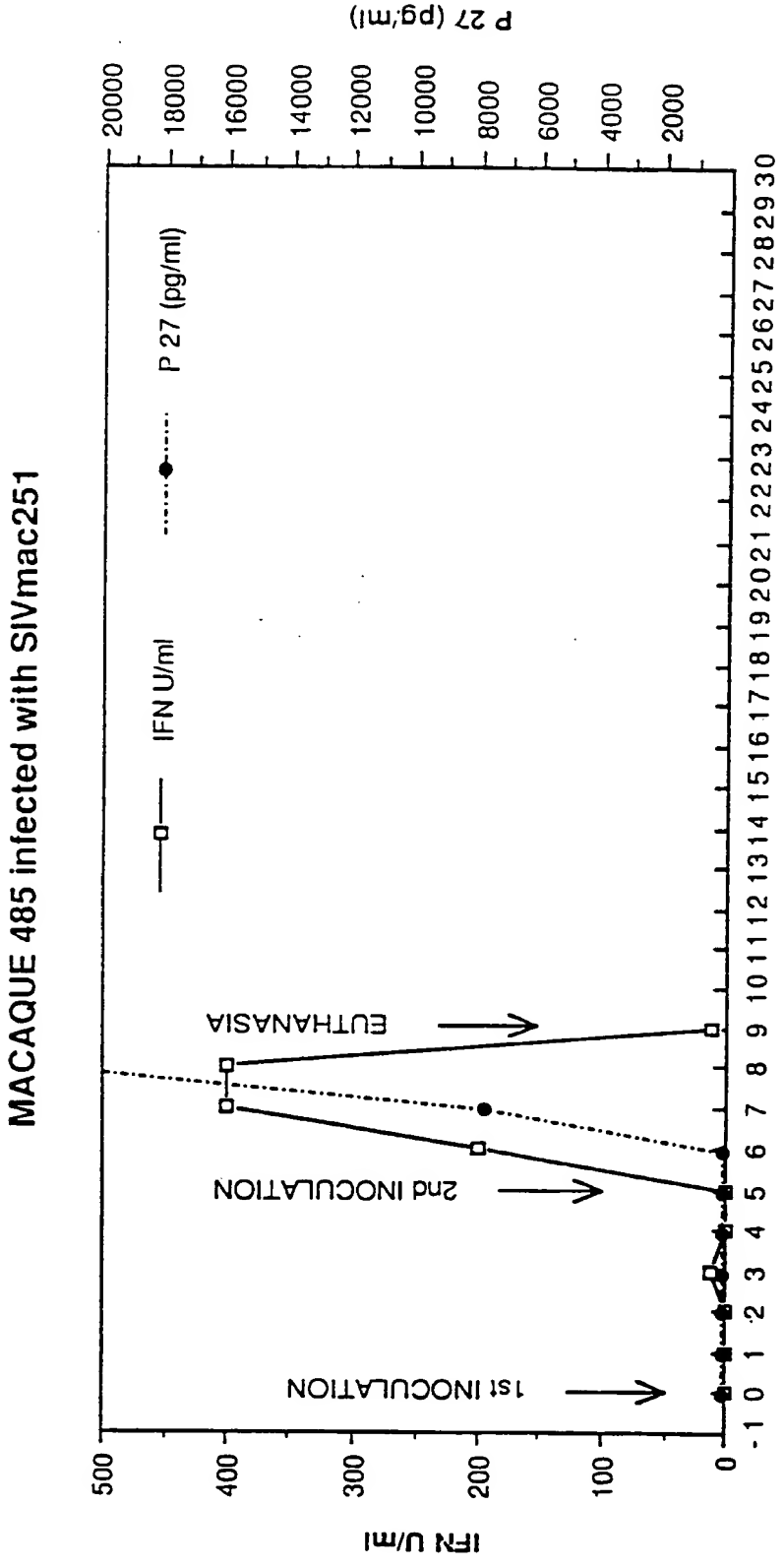

MACAQUE 485 infected with SIVmac251
IFN U/ml
P 27 (pg/ml)
1st INOCULATION
2nd INOCULATION
EUTHANASIA
MONTHS POST INFECTION
0
100
200
300
400
500
2000
4000
6000
8000
10000
12000
14000
16000
18000
20000
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

FIGURE 10

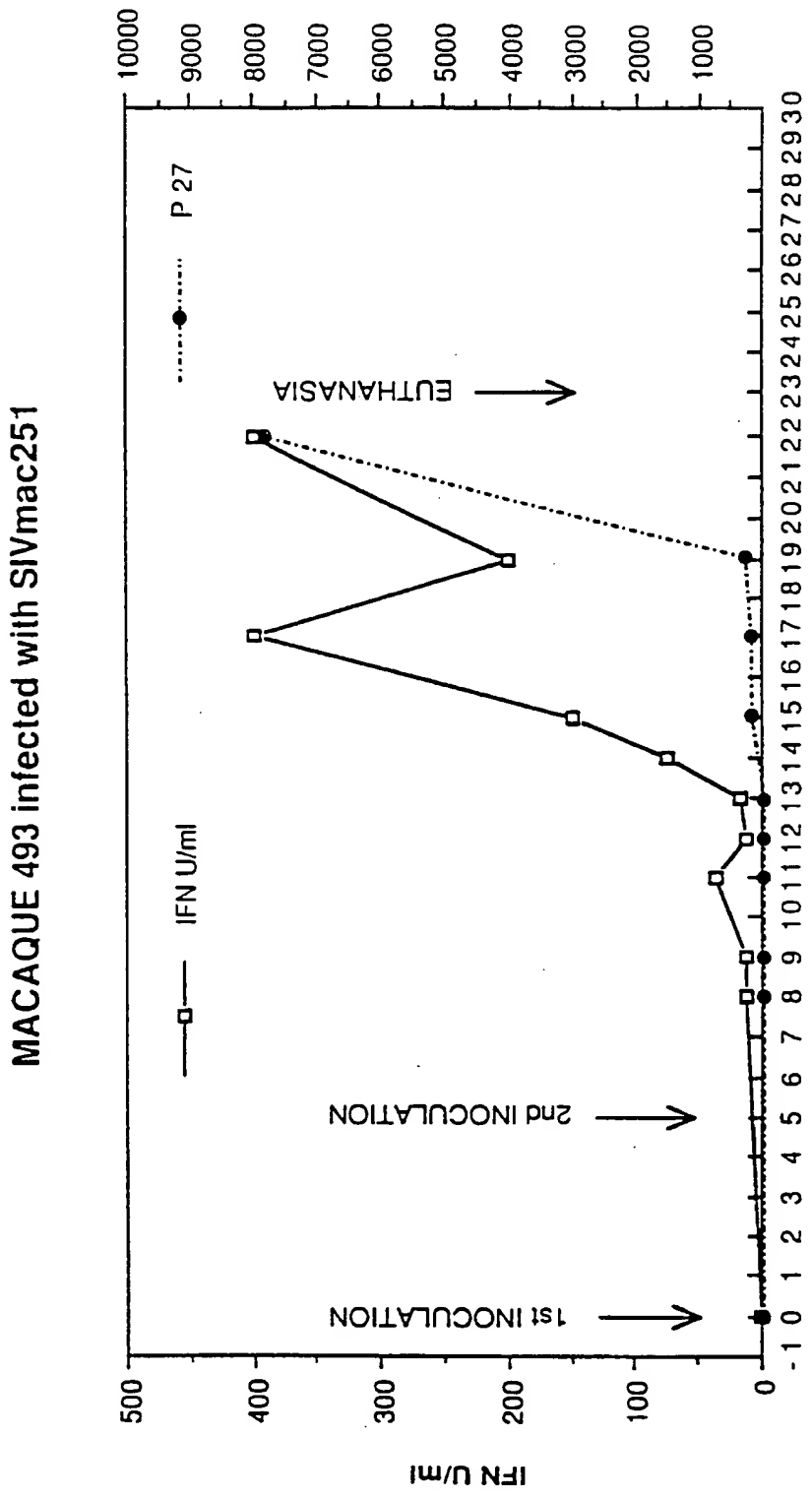

MACAQUE 493 infected with SIVmac251
IFN U/ml
P 27 (pg/ml)
IFN U/ml
P 27
1st INOCULATION
2nd INOCULATION
EUTHANASIA
MONTHS POST INFECTION
0
100
200
300
400
500
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

FIGURE 11

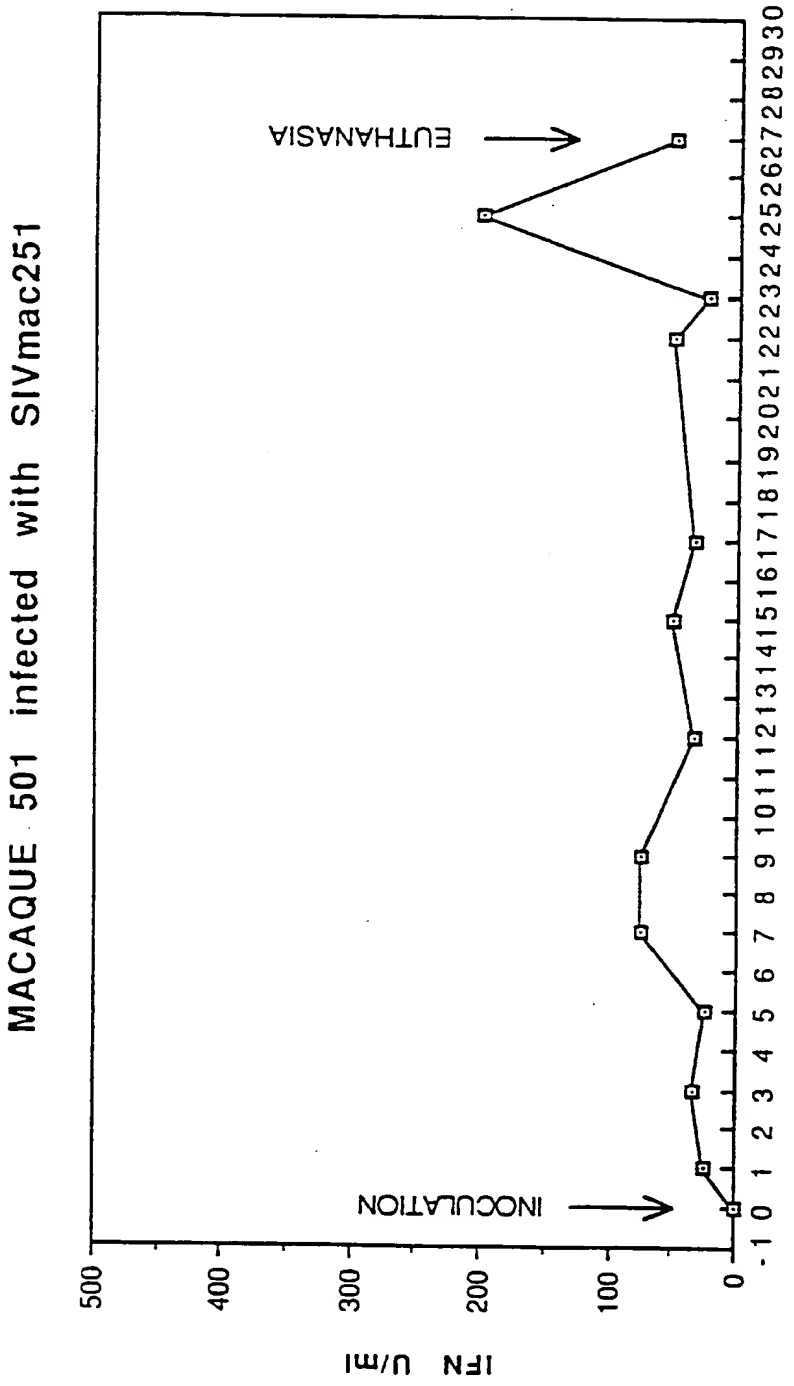

MACAQUE 501 infected with SIVmac251
EUTHANASIA
INOCULATION
IFN U/ml
0
100
200
300
400
500
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
MONTHS POST INFECTION

FIGURE 12

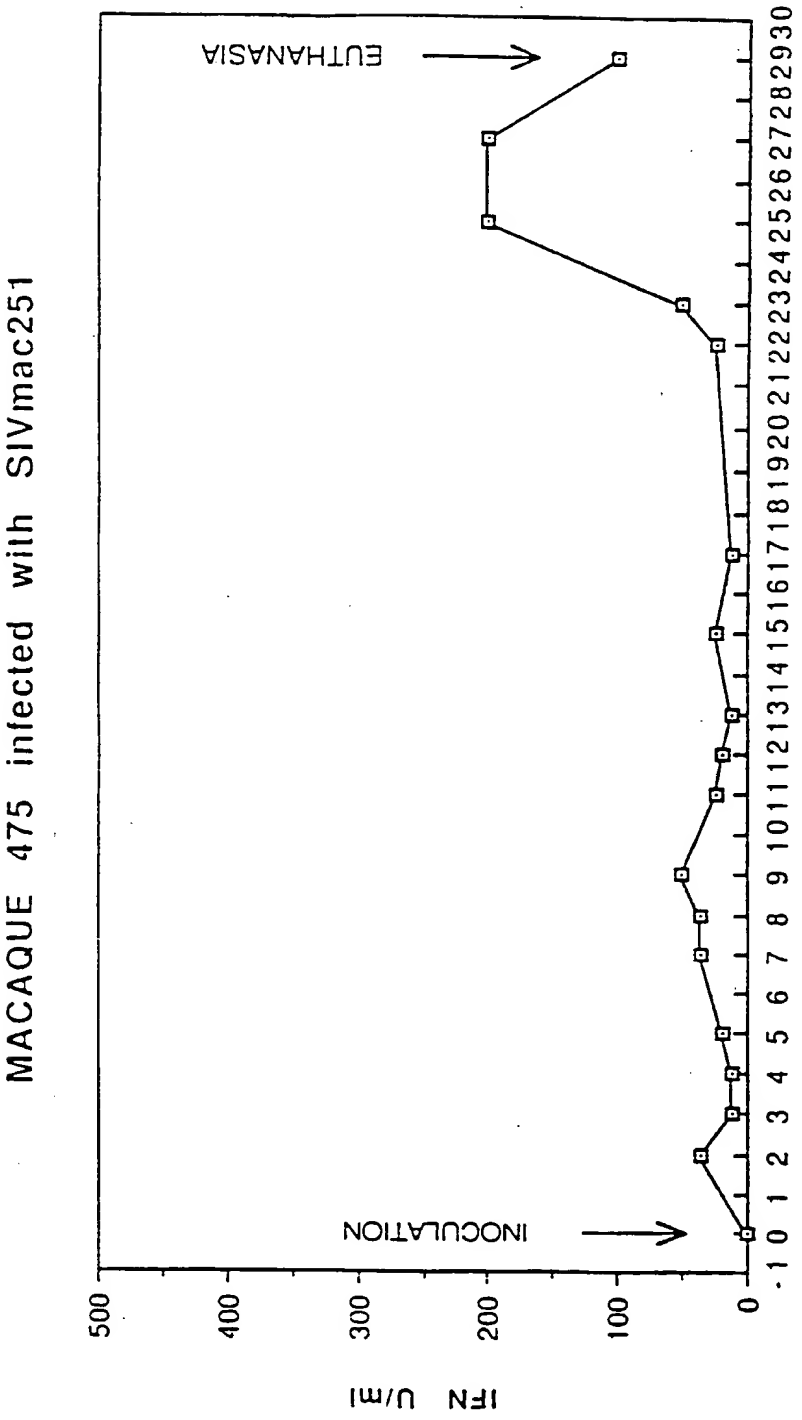

MACAQUE 475 infected with SIVmac251
EUTHANASIA
INOCULATION
IFN U/ml
500
400
300
200
100
0
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
MONTHS POST INFECTION

FIGURE 13

ELISA FOR THE DETECTION OF ANTI HUMAN IFN α RECEPTOR ANTIBODY

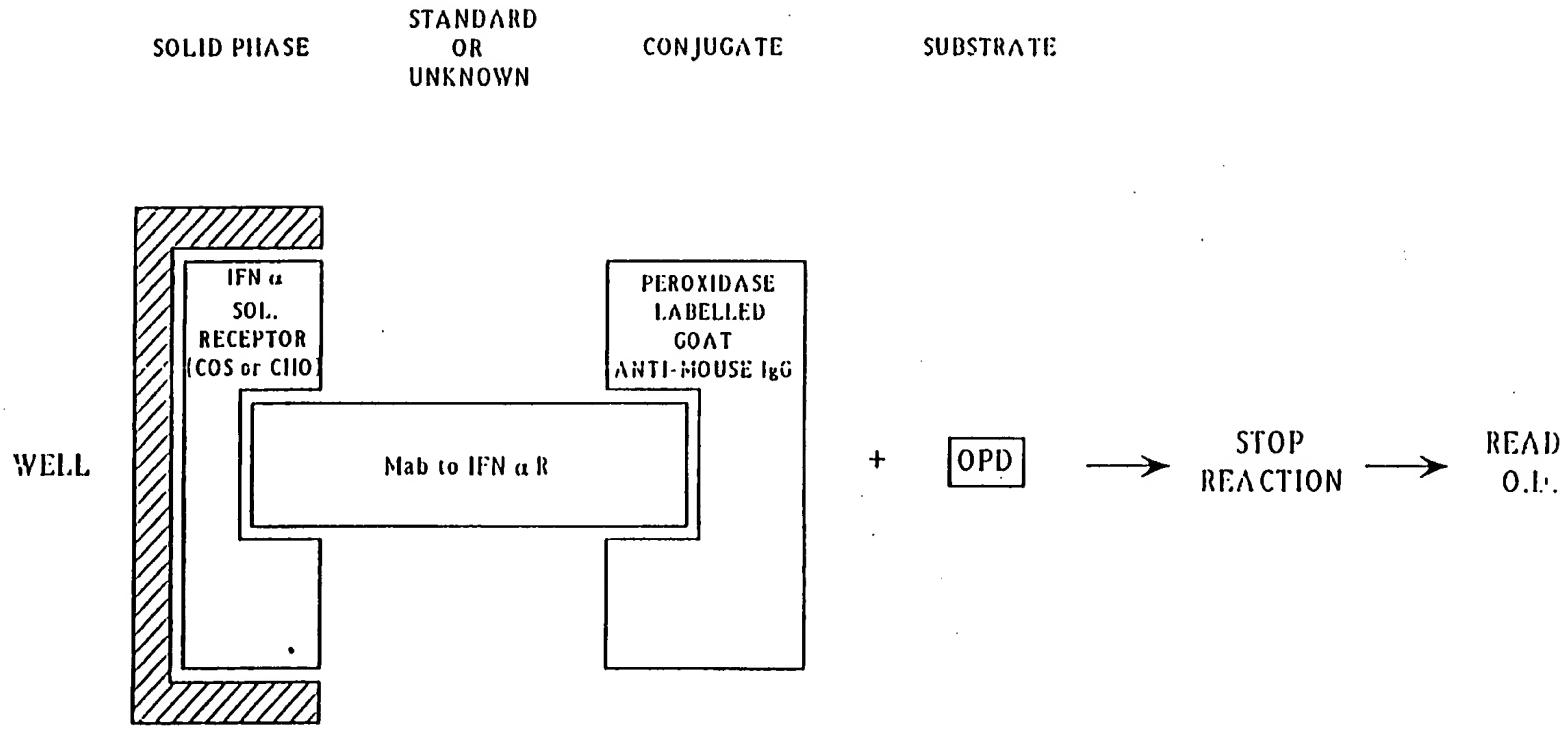


FIGURE 14(1)

ELISA FOR THE DETECTION OF HUMAN IFN α SOLUBLE RECEPTOR

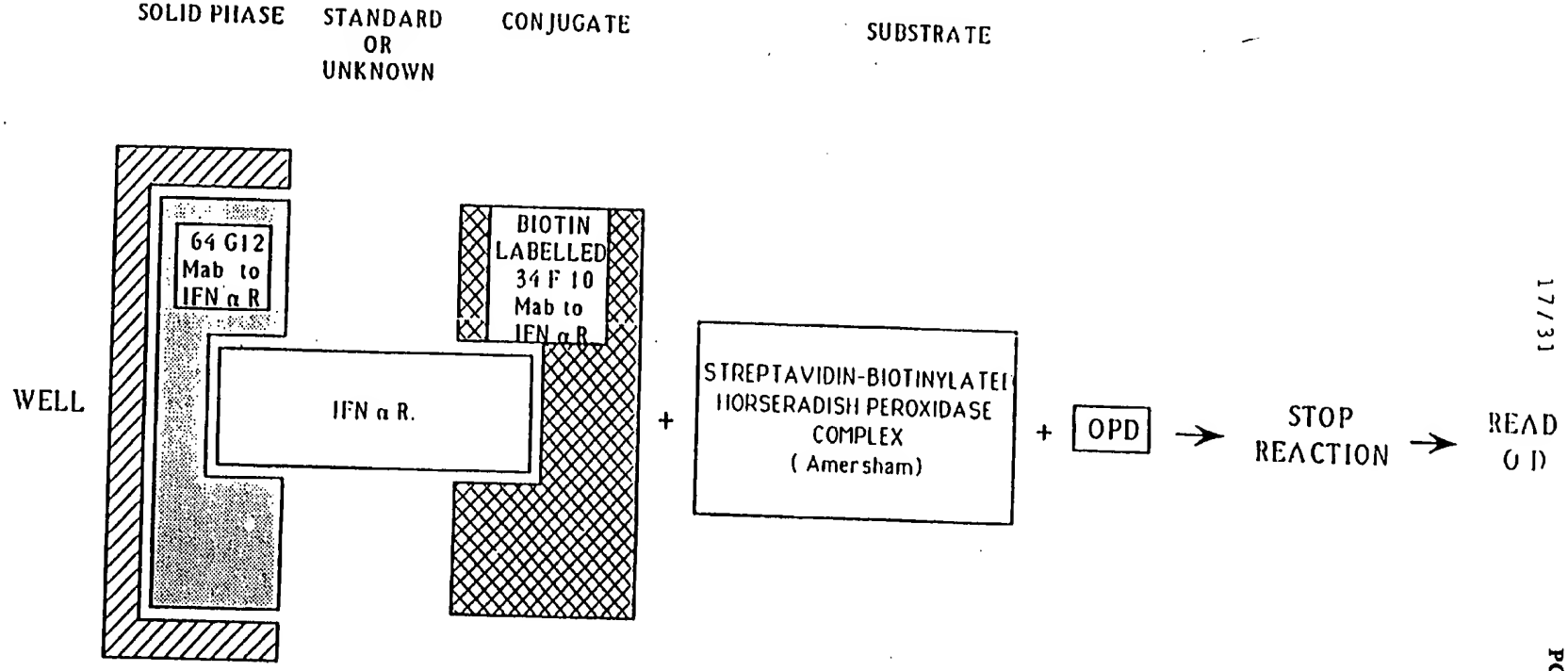


FIGURE 14(2)

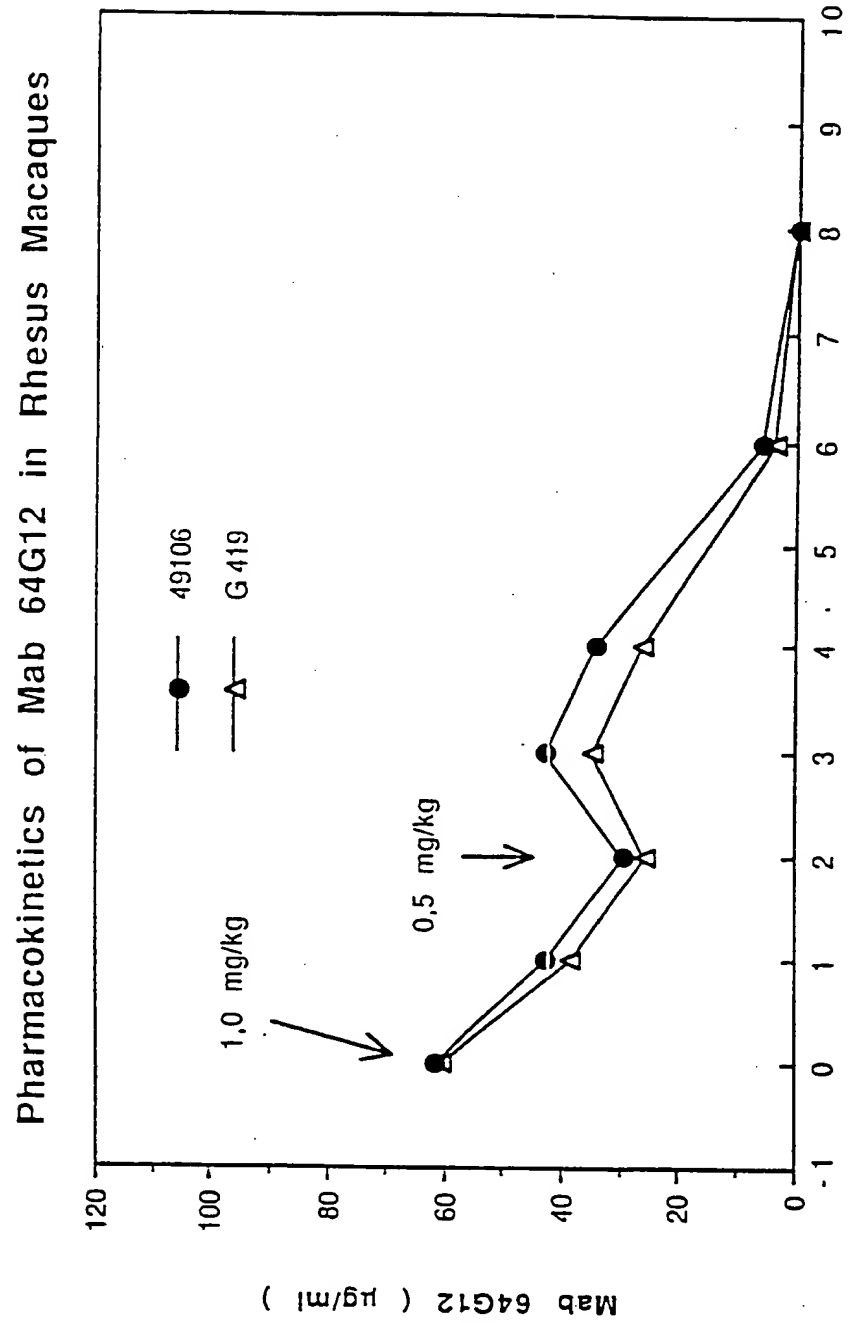
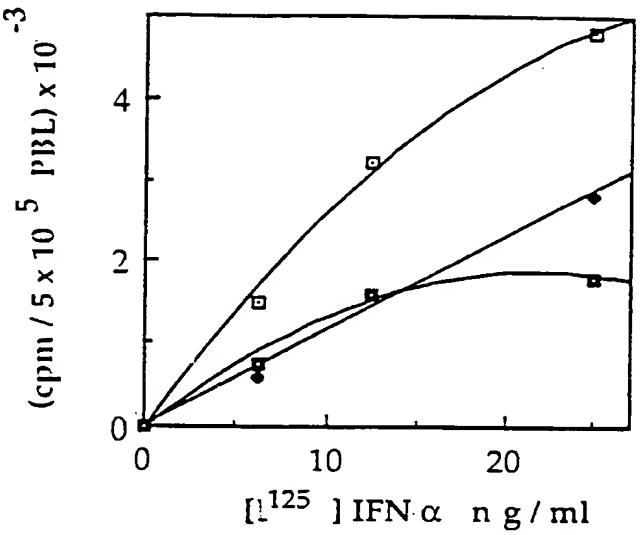

Pharmacokinetics of Mab 64G12 in Rhesus Macaques
49106
G 419
1,0 mg/kg
0,5 mg/kg
Mab 64G12 (µg/ml)
TIME in days
120
100
80
60
40
20
0
-1
0
1
2
3
4
5
6
7
8
9
10

FIGURE 15


(cpm / 5 x 10 5 PBL) x 10 -3
4
2
0
0
10
20
[I125] IFN α n g / ml

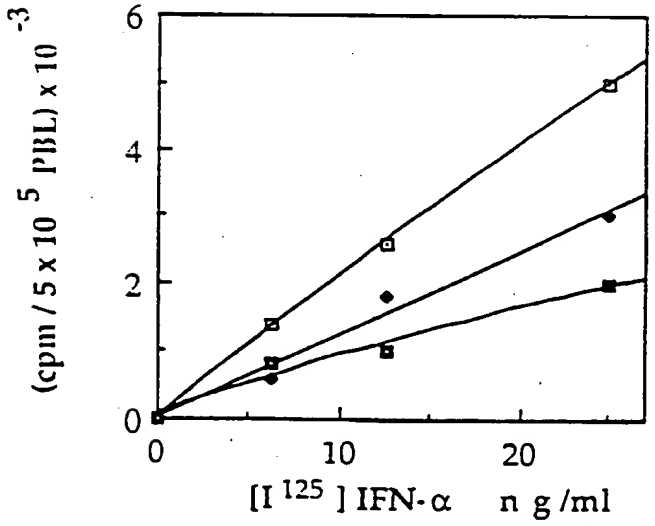
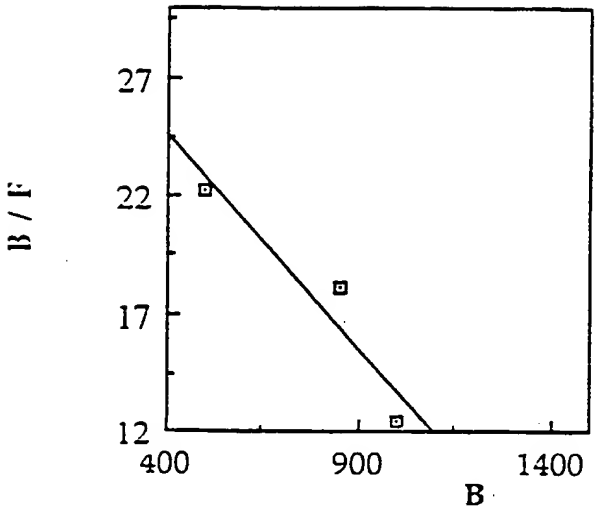

(cpm / 5 x 10 5 PBL) x 10 -3
6
4
2
0
0
10
20
[I 125] IFN-α n g /ml

FIGURE 16



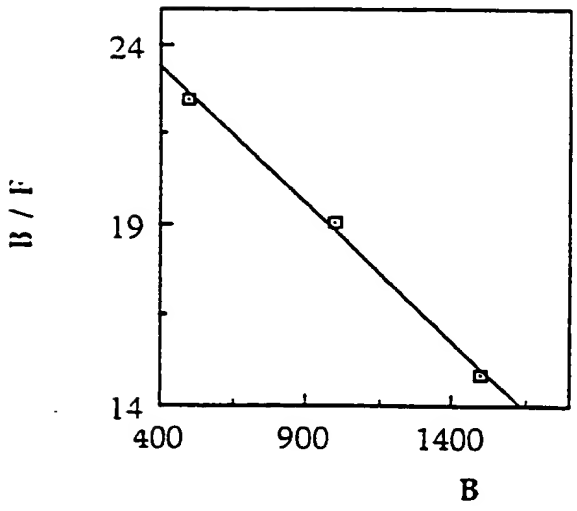
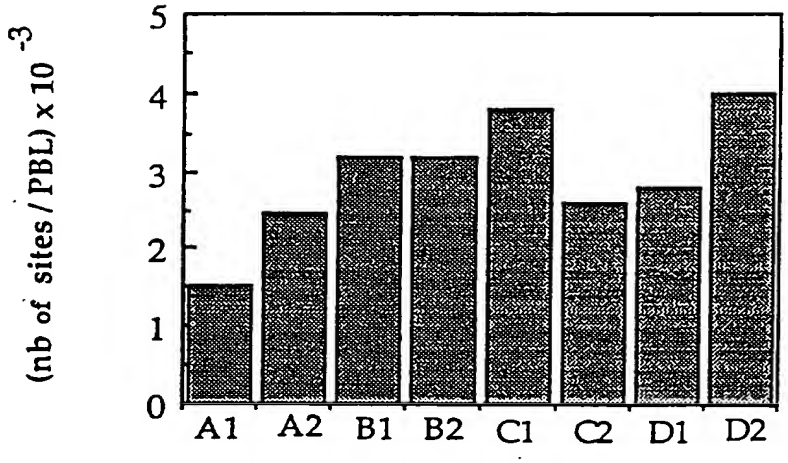


FIGURE 17



Kd = 2-8 x 10 $^{-10}$M

FIGURE 18

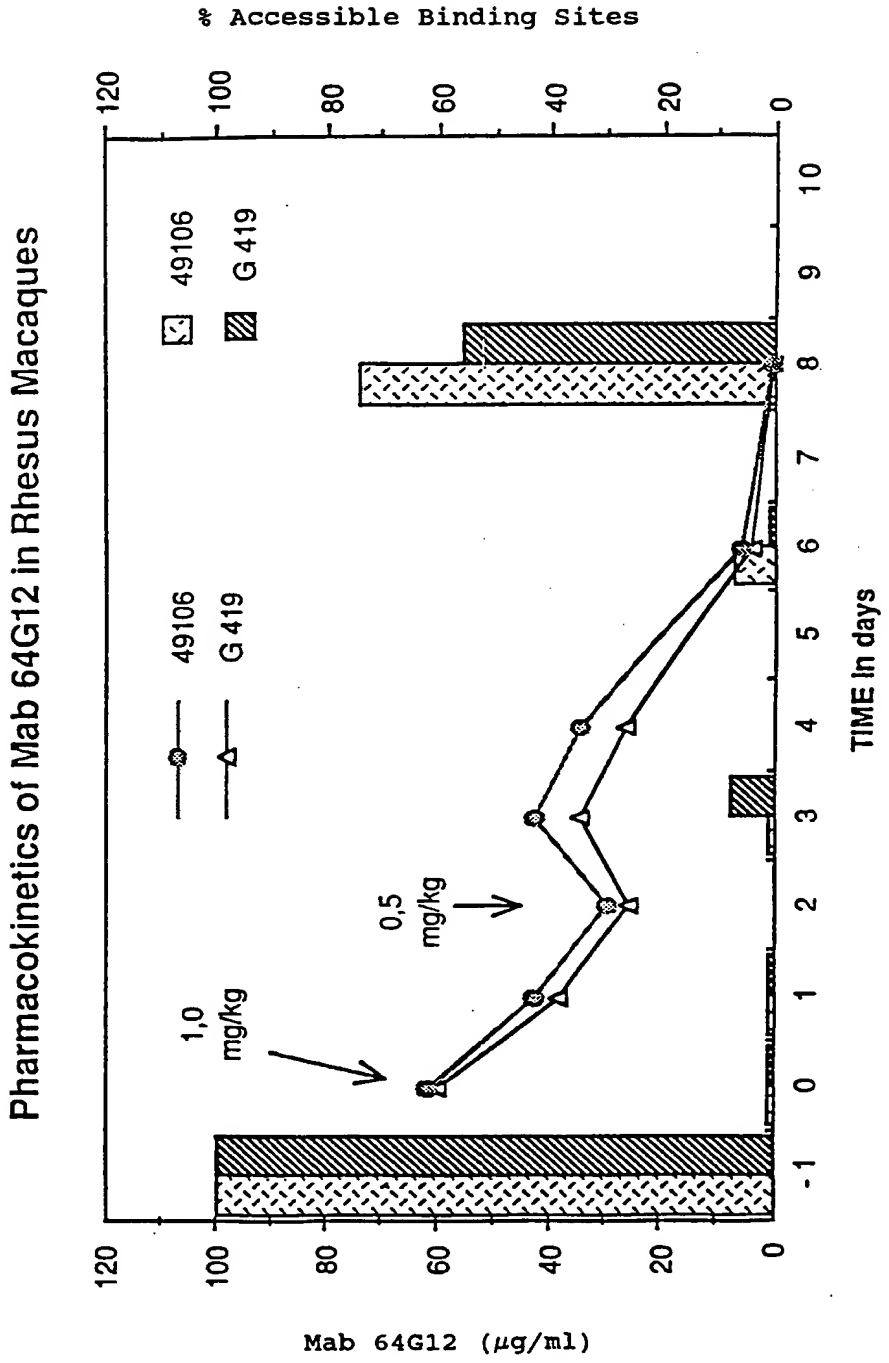

% Accessible Binding Sites
Pharmacokinetics of Mab 64G12 in Rhesus Macaques
49106
G 419
49106
G 419
1,0 mg/kg
0,5 mg/kg
TIME in days
Mab 64G12 (µg/ml)

FIGURE 19

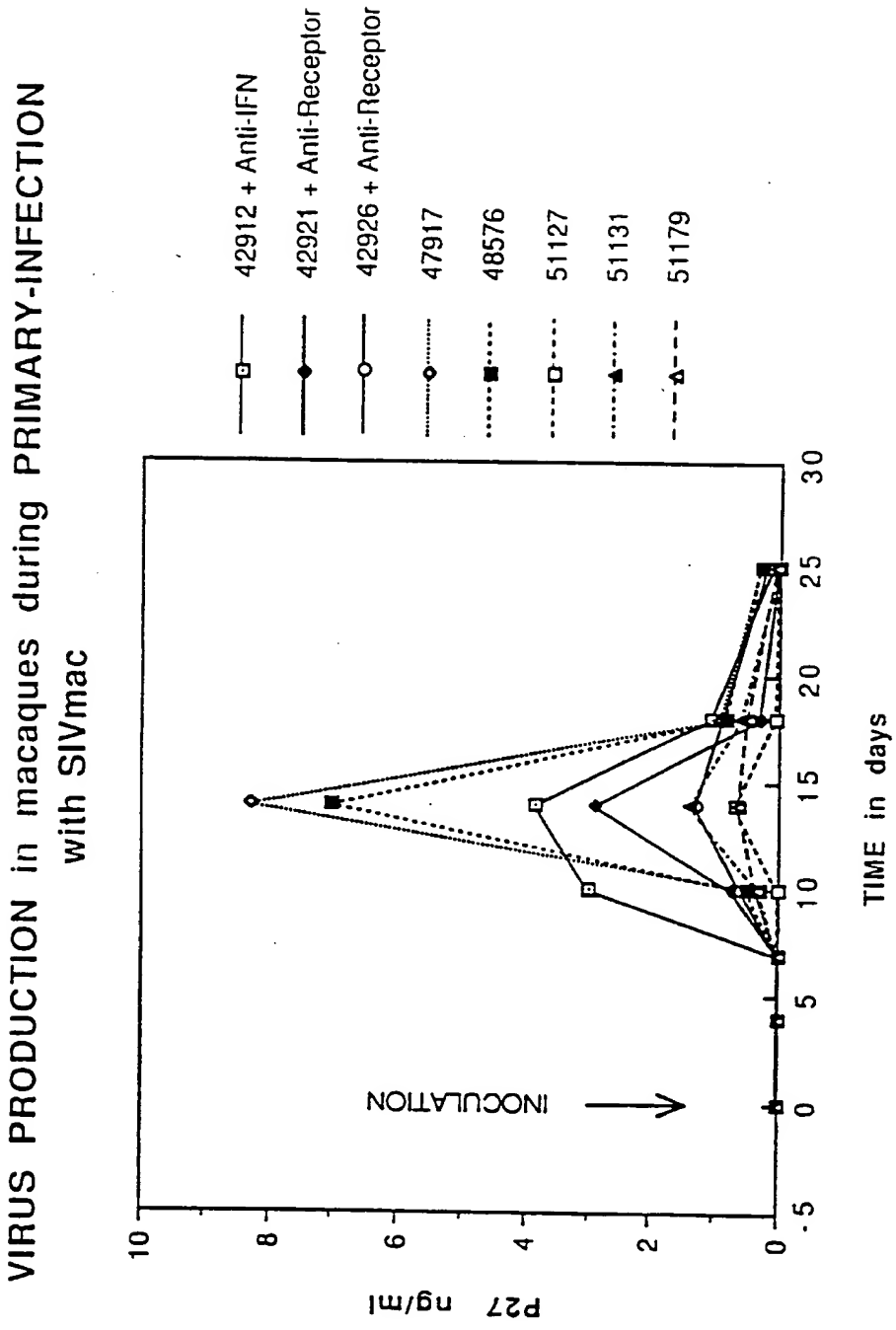

VIRUS PRODUCTION in macaques during PRIMARY-INFECTION
with SIVmac
42912 + Anti-IFN
42921 + Anti-Receptor
42926 + Anti-Receptor
47917
48576
51127
51131
51179
INOCULATION
P27 ng/ml
TIME in days
0
2
4
6
8
10
-5
0
5
10
15
20
25
30

FIGURE 20

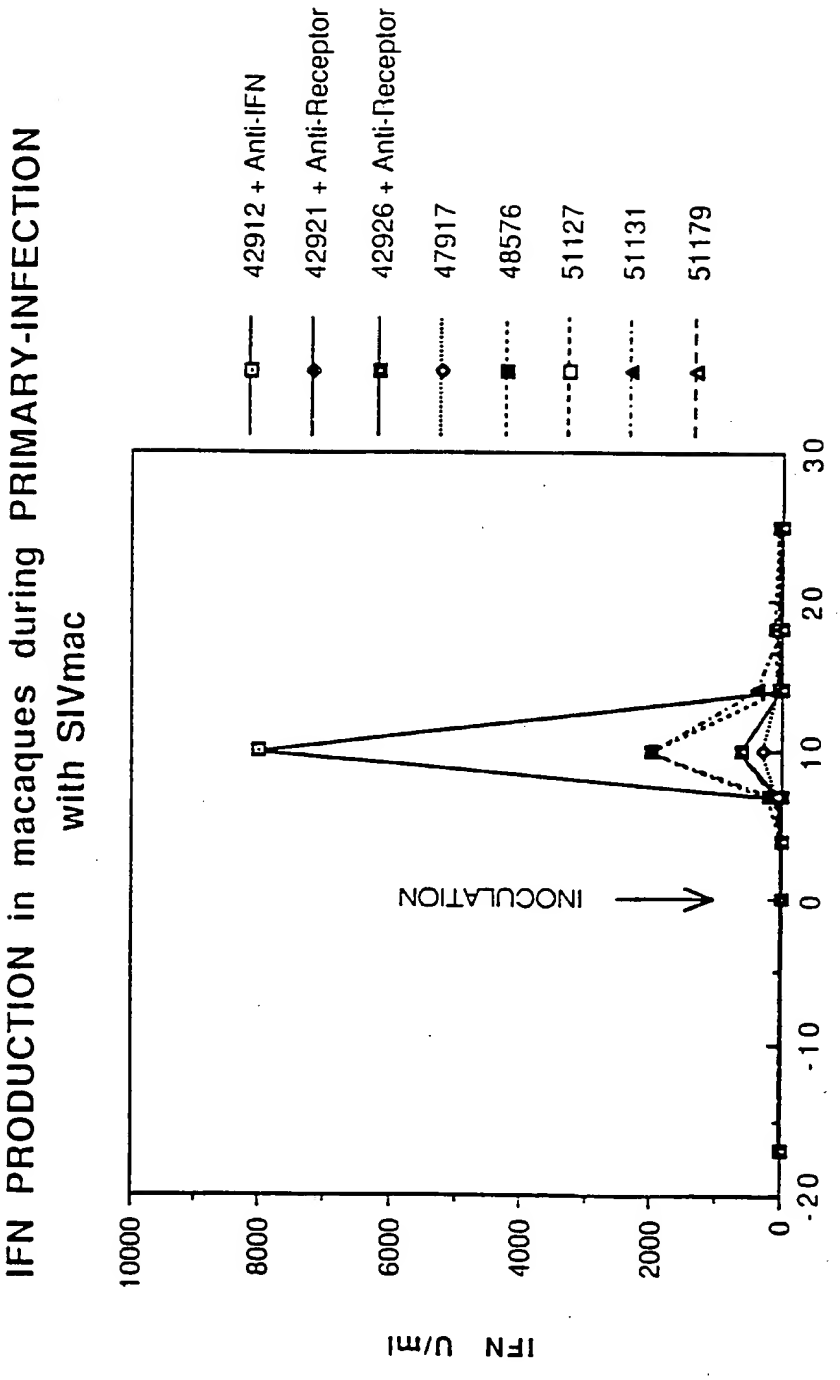

IFN PRODUCTION in macaques during PRIMARY-INFECTION
with SIVmac
42912 + Anti-IFN
42921 + Anti-Receptor
42926 + Anti-Receptor
47917
48576
51127
51131
51179
INOCULATION
IFN U/ml
TIME in days
10000
8000
6000
4000
2000
0
-20
-10
0
10
20
30

FIGURE 21

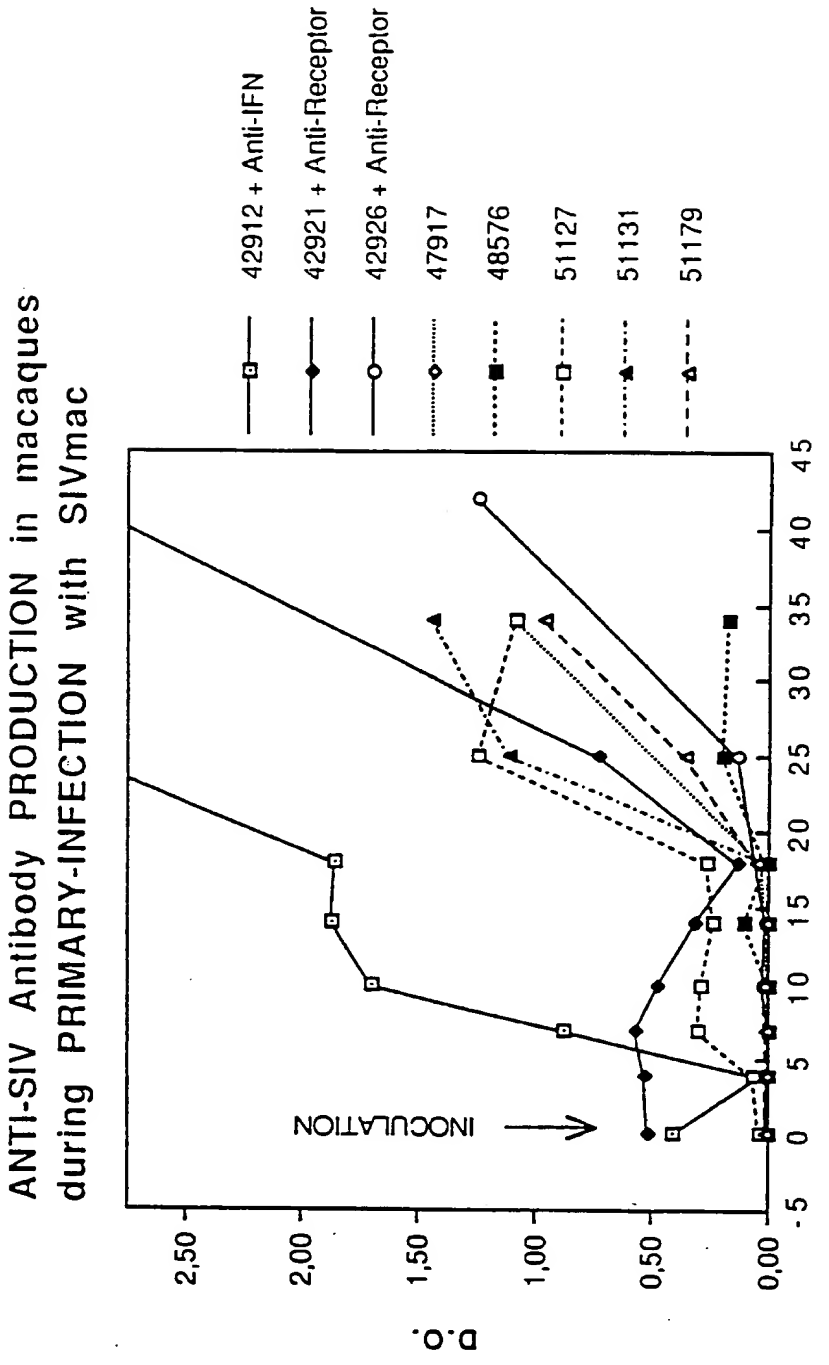

ANTI-SIV Antibody PRODUCTION in macaques
during PRIMARY-INFECTION with SIVmac
42912 + Anti-IFN
42921 + Anti-Receptor
42926 + Anti-Receptor
47917
48576
51127
51131
51179
INOCULATION
O.D.
2,50
2,00
1,50
1,00
0,50
0,00
-5
0
5
10
15
20
25
30
35
40
45
TIME in days

FIGURE 22

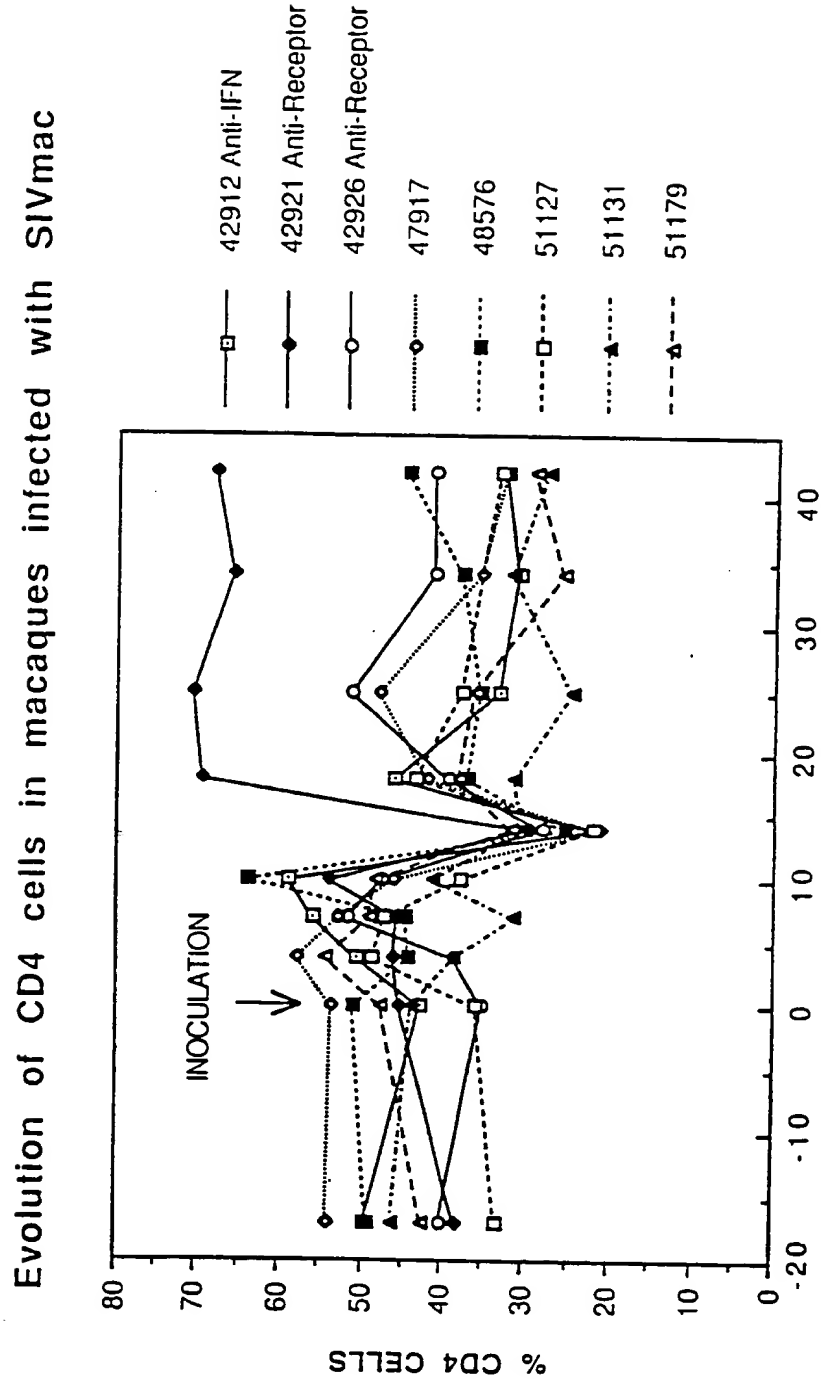

Evolution of CD4 cells in macaques infected with SIVmac
42912 Anti-IFN
42921 Anti-Receptor
42926 Anti-Receptor
47917
48576
51127
51131
51179
INOCULATION
% CD4 CELLS
TIME in days
80
70
60
50
40
30
20
10
0
-20
-10
0
10
20
30
40

FIGURE 23

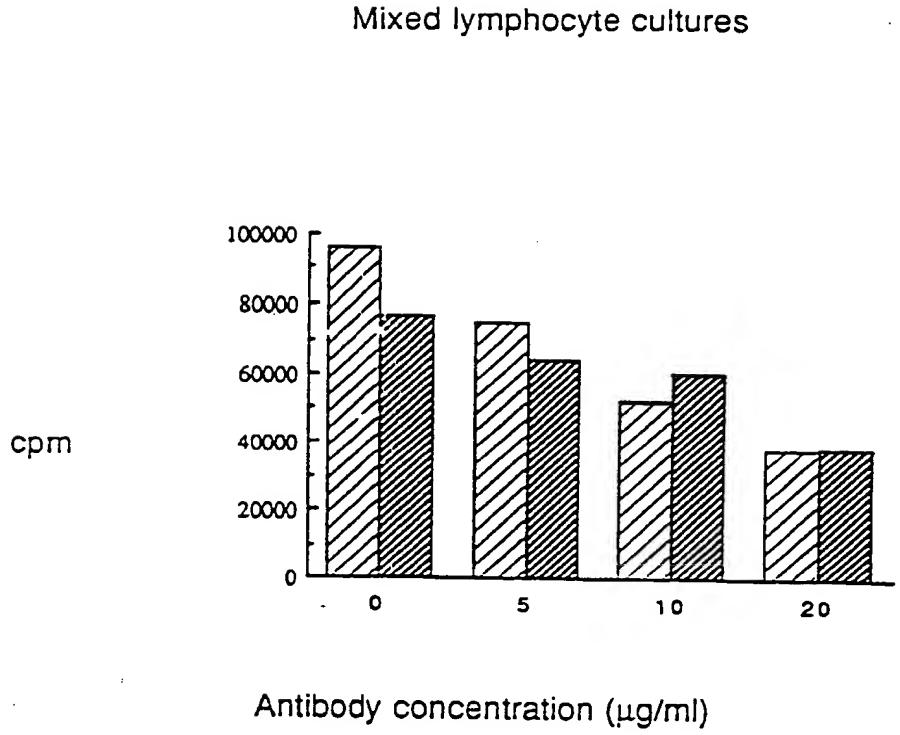
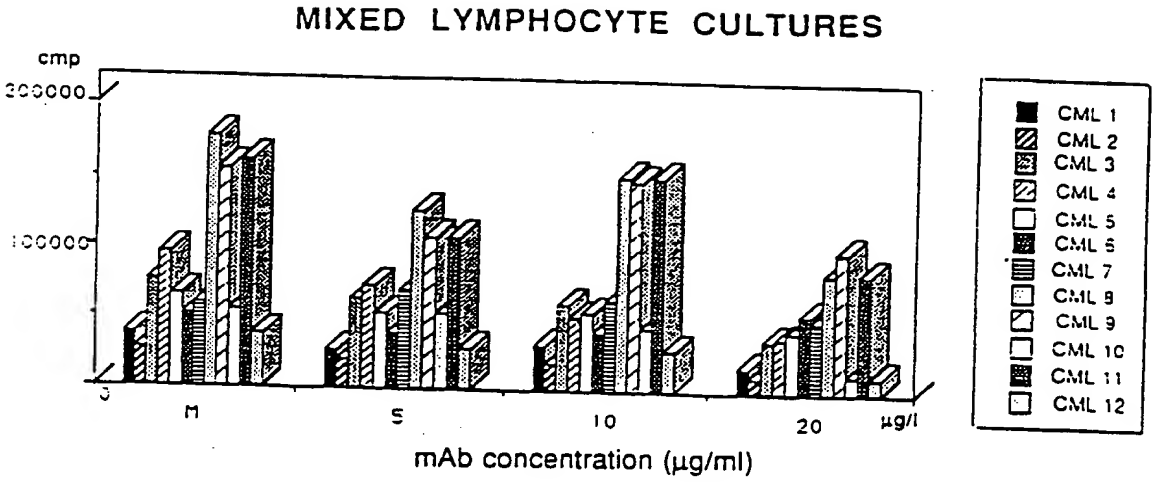

Mixed lymphocyte cultures
cpm
100000
80000
60000
40000
20000
0
0
5
10
20
Antibody concentration (µg/ml)

FIGURE 24



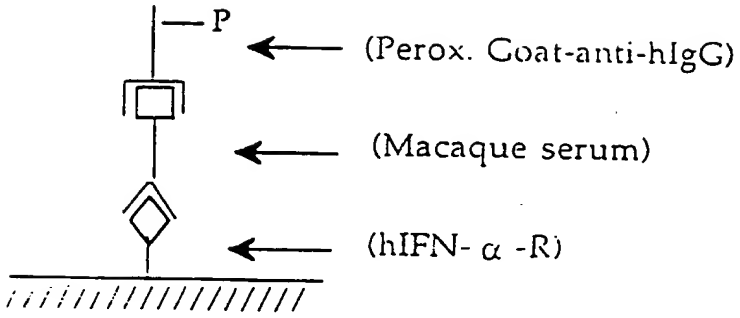
% inhibition of MLR induced by mAb 64G12 (20 µg/ml)

CML	%
1	29
2	71
3	74
4	82
5	68
6	44
7	20
8	49
9	60
10	39
11	39
12	32

The inhibition of 3H dTR incorporation varied between 20 and 82 % in the presence of 20 µg/ml of mAb 64G12

FIGURE 25

A


P
(Perox. Goat-anti-hIgG)
(Macaque serum)
(hIFN- α -R)

B

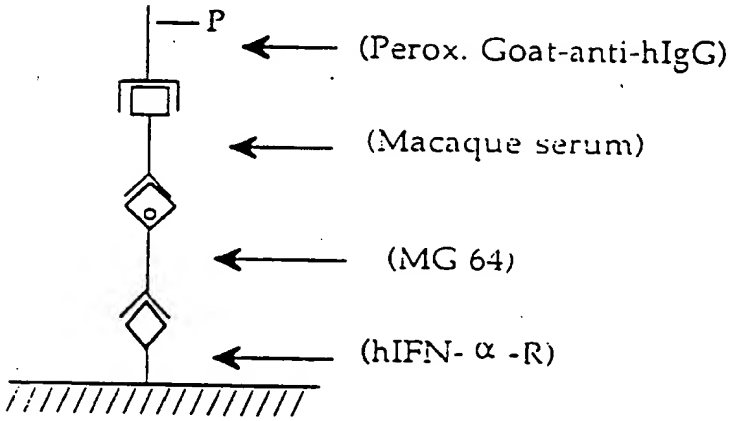
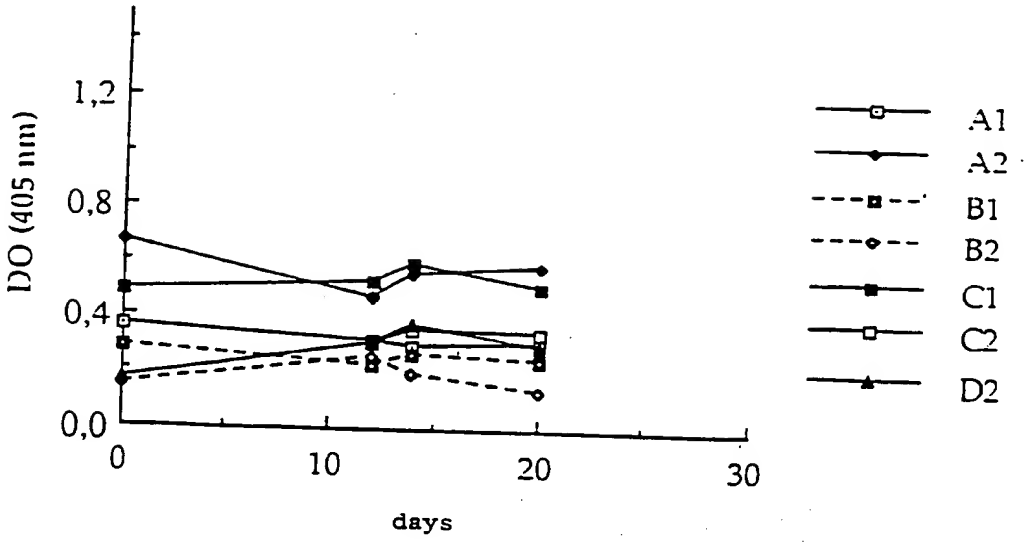

P
(Perox. Goat-anti-hIgG)
(Macaque serum)
(MG 64)
(hIFN- α -R)

FIGURE 26


DO (405 nm)
1,2
0,8
0,4
0,0
0
10
20
30
days
A1
A2
B1
B2
C1
C2
D2

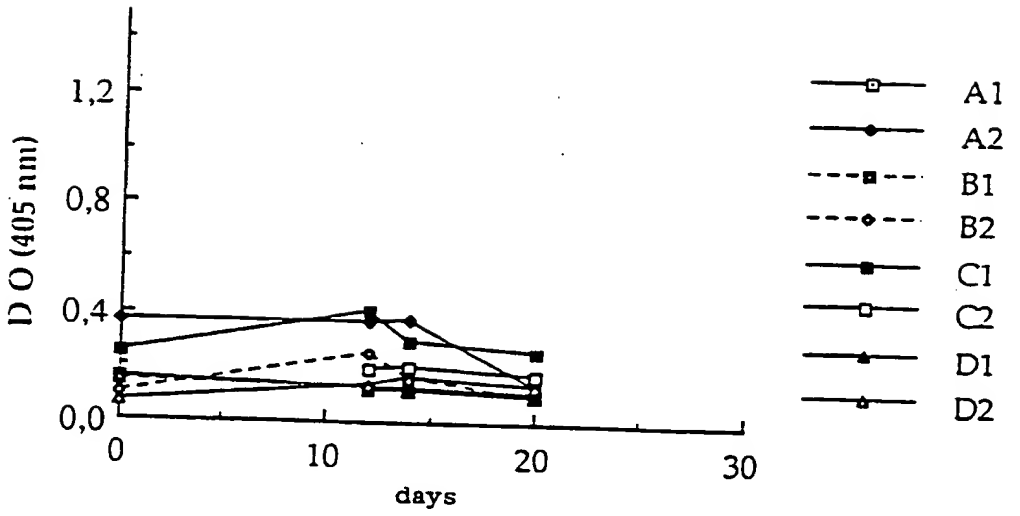
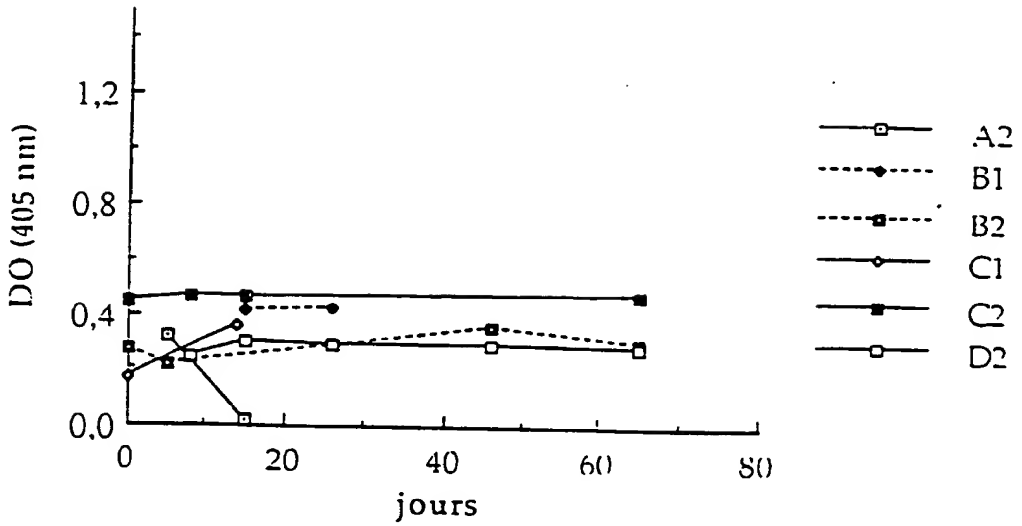

D O (405 nm)
1,2
0,8
0,4
0,0
0
10
20
30
days
A1
A2
B1
B2
C1
C2
D1
D2

FIGURE 27

A


DO (405 nm)
1,2
0,8
0,4
0,0
0
20
40
60
80
jours
A2
B1
B2
C1
C2
D2

B

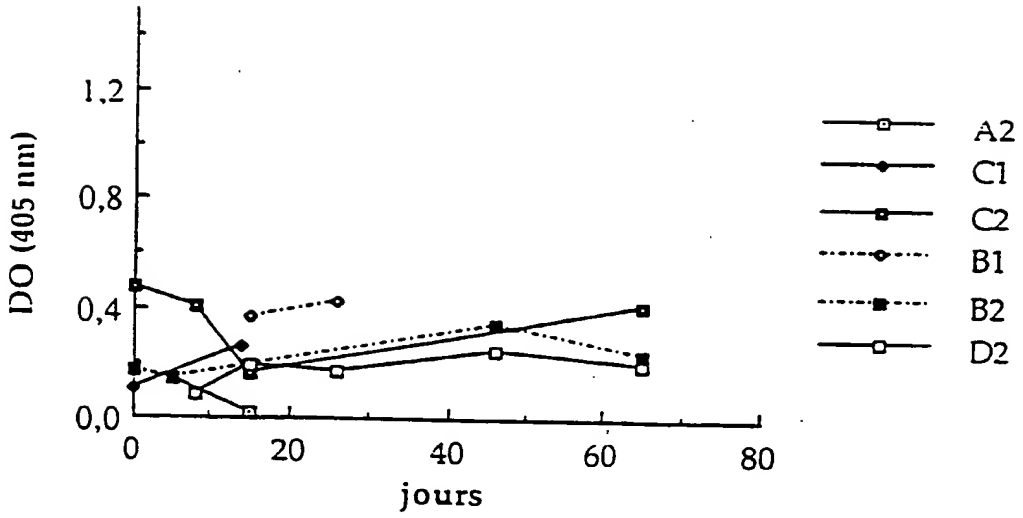

DO (405 nm)
1,2
0,8
0,4
0,0
0
20
40
60
80
jours
A2
C1
C2
B1
B2
D2

FIGURE 28

INTERNATIONAL SEARCH REPORT

I ational Application No
PCT/EP 94/03114

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/395 G01N33/577 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	THE JOURNAL OF IMMUNOLOGY, vol.150, no.3, 1 February 1993, BALTIMORE MD, USA pages 707 - 716 P. BENOIT ET AL. 'A monoclonal antibody to recombinant human IFN-alpha receptor inhibits biologic activity of several species of human IFN-alpha, IFN-beta, and IFN-omega.' see the whole document --- -/--	1-28

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 January 1995	23 -01- 1995

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Nooij, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 94/03114

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF INTERFERON RESEARCH, vol.13, no.4, August 1993, NEW YORK, USA pages 295 - 301 J. LIM ET AL. 'Generation and characterization of anti-idiotypic antibodies recognizing the interferon-alpha receptor: Implications for ligand-receptor interactions.' see the whole document	1,2,5,6, 8,10,12, 13,28
X	WO,A,93 04699 (GENENTECH, INC.) 18 March 1993 see page 7, line 36 - page 9, line 9 see claims 1,2,13,14	1-4,8, 15,16,28
A	EP,A,0 369 877 (YEDA RESEARCH AND DEVELOPMENT COMPANY LIMITED) 23 May 1990 see page 7, line 28 - page 8, line 3	1
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol.87, September 1990, WASHINGTON DC, USA pages 7230 - 7234 O. COLAMONICI ET AL. 'Characterization of three monoclonal antibodies that recognize the interferon alpha2 receptor.' see the whole document	1
A	THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol.267, no.33, 25 November 1992, BALTIMORE MD, USA pages 24053 - 24057 L. PLATANIAS ET AL. 'Interferon alpha induces rapid tyrosine phosphorylation of the alpha subunit of its receptor.' see the whole document	1
A	WO,A,91 05862 (CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE (CNRS)) 2 May 1991 see claims	1
P,X	EP,A,0 563 487 (LABORATOIRE EUROPEEN DE BIOTECHNOLOGIE S.A.) 6 October 1993 see the whole document	1-28

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 94/03114

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9304699	18-03-93	EP-A-	0601052	15-06-94
		JP-T-	6510061	10-11-94
EP-A-0369877	23-05-90	AU-B-	628895	24-09-92
		AU-A-	4666189	12-06-90
		CA-A-	2002862	14-05-90
		WO-A-	9005737	31-05-90
WO-A-9105862	02-05-91	FR-A-	2653445	26-04-91
		EP-A-	0495907	29-07-92
		JP-T-	5504468	15-07-93
EP-A-0563487	06-10-93	AU-B-	3890793	08-11-93
		WO-A-	9320187	14-10-93
		NO-A-	943625	11-11-94